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**14. ABSTRACT**

During the first year of the grant, one paper was accepted for publication based on the grantees PhD work. This paper would not have been completed without the additional resources provided by this grant and the grant is duly acknowledged in the paper. One other paper was published in the year as a result of collaborations with some of the grantees mentors. Both papers examined differences in genetic polymorphisms in prostate cancer by race/ethnicity in a cohort of men from south Texas. Further research is underway as a panel of new SNPs has been chosen and samples are currently being genotyped. Genotyping should be completed by mid 2008. The planned sociodemographic survey has been delayed but is currently being developed and should be completed within the next funding year. The grantees health disparities training program is going well. The grantee has taken two classes related to her work (Chronic Disease Epidemiology and Analytic Epidemiology) and is currently teaching a graduate level introductory epidemiology class with a focus on studying health disparities. She attended the "Science of Health Disparities" conference in Atlanta, GA, from November 27-30, 2007.

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# Annual Report (Year 1 of 3): March 2008

**Contract #: W81XWH-07-1-0234**
**Grant#: PC060447**
**PI: Kathleen C. Torkko, PhD, MSPH**
**Title: Ethnicity and Prostate Cancer: Vitamin D Genetic and Sociodemographic Factors**

## Introduction

The main purposes of this grant were to provide opportunities for the principle investigator to expand her PhD work and to receive training in cancer health disparity research, specifically in prostate cancer. This training program involves meeting with mentors for guidance, taking classes pertinent to her research and training objectives, and attending appropriate conferences.

Her research work is on differences in vitamin D receptor (*VDR*) genetic relationships to prostate cancer between non-Hispanic White (NHW) and Hispanic White (HW; mainly of Mexican origin) men in the SABOR (San Antonio Biomarkers Of Risk for prostate cancer) study run by researchers at the University of Texas Health Sciences Center, San Antonio (UTHSCSA). Hispanic men have been a focus of Dr. Torkko's PhD research for two reasons: they comprise the largest minority population in the SABOR study, and they are a largely understudied population in prostate cancer and genetic epidemiology. Understanding the relationship of genetics to prostate cancer and how this relationship varies by race/ethnicity can help elucidate racial differences seen in prostate cancer diagnosis, treatment, and survival.

This grant allows Dr. Torkko to increase the number of study participants and the number of genes and genetic polymorphisms studied to examine the effects on risk for prostate cancer by ethnicity of gene-gene interactions between the vitamin D receptor (*VDR*) gene and other genes in the metabolic pathway of vitamin D and testosterone.

Another objective of this proposal is to determine if sociodemographic factors differ between NHW, HW, and potentially African American men (if numbers increase) in the SABOR study and if a relationship exists between sociodemographic and genetic factors. This will be accomplished by developing and conducting a sociodemographic survey in the SABOR population.

The support provided by this Traineeship award is providing Dr. Torkko opportunities to develop as an independent prostate cancer epidemiology researcher and to further develop her areas of expertise by providing opportunities to explore differences in prostate cancer by race/ethnicity.

## Body

### Prostate Cancer Training Program Progress

Mentorship/Collaborations

Dr. Torkko has met with the members of her mentorship panel who have provided guidance on how to get her PhD work published and in the development of current projects. Dr. Scott Lucia, the primary mentor, an expert prostate cancer pathologist, employs Dr. Torkko as an epidemiologist/statistician in the Prostate Cancer Research Laboratory (PCRL) in the Department of Pathology at the University of Colorado Denver (UCD). Dr. Lucia has facilitated Dr. Torkko's involvement in the development of a cancer biorepository at UCD. Dr. Torkko will assist in building the patient database and developing research using available resources. This will hopefully lead to other funding opportunities. Dr. Lucia is also providing opportunities for first authorship on a paper involving research projects in the PCRL. Meetings with Dr. Lucia and other mentors have involved discussions of future projects and funding opportunities. Meetings with Dr. Robin Leach and Dr. Ian Thompson during a visit to San Antonio, TX, in April 2007, resulted in more data collection and genetic analyses that enhanced her PhD work and led to a publication on genetic differences by ethnicity in gene-gene interactions in the *VDR* and *SRD5A2* genes [Torkko KC *et al. Clinical Cancer Research* (in press)]. Dr. Torkko also collaborated with her Texas colleagues in the publication of a paper on *RNASEL* variants and

their associations with prostate cancer in ethnic/racial minorities in the SABOR cohort (Shook SJ e*t al., Clin Cancer Res* 2007; 13;5959-64).

Scientific Conferences

As part of the training for the grant, Dr. Torkko is expected to attend scientific conferences chosen to be relevant to prostate cancer, genetic epidemiology, and/or health disparity/cultural competency. Funds have been allocated to attend at least one conference each funded year. In the 2007-08 grant period, Dr. Torkko attended the first American Association of Cancer Researchers (AACR) conference on "The Science of Cancer Health Disparities in Racial/Ethnic Minorities and Medically Underserved" in Atlanta, GA, from November 27-30, 2007. As research data become available, it is expected that abstracts will be submitted for future conferences.

Coursework

As part of the training for the grant, Dr. Torkko is expected to continue her education by taking classes on epidemiology and cultural issues. Dr. Torkko took two classes within the timeframe of the first year of the grant. In the spring semester 2007, she took the Chronic Disease Epidemiology class (PRMS 6636) offered by the Department of Preventive Medicine and Biometrics to refresh her knowledge of epidemiologic techniques. As part of the class, students were required to write a proposal using life-course methodology. Dr. Torkko wrote a proposal titled: "A Life-Course Analytical Approach for Understanding Early Exposure to Androgens and Risk for Prostate Cancer: A Case-Control Study in a Multiethnic Cohort from South Texas." It was her aim to design a proposal that would examine exposures early in life that may explain differences in prostate cancer incidence seen between Non-Hispanic Whites and Hispanic Whites. She would like to propose this study in the SABOR cohort, but would need to procure some additional funding for this effort. The proposal is attached in the Appendix. She received an "A" in the class (see transcripts in Appendix).

In the fall semester 2007, she took an analytic epidemiology class (PRMD 7915) on survey design. She had hoped to use this class to help design the sociodemographic survey for the SABOR population. Unfortunately, the professor for the class changed the content of the class at the last moment from what was advertised to cover complex sampling designs which, although interesting, ended up having no real relationship to her research. (she received an "A" in the class). To facilitate the development of the study, she has started collaborating with a former professor who assisted with her Masters work (where she conducted a survey of Colorado primary care providers) to help design the survey to be developed and administered in the next funding year.

In the spring semester 2008, rather than taking a class, Dr. Torkko is teaching the Introductory Epidemiology class (HBSC 4001/5001) for the Health and Behavior Sciences Department at the UCD. She made health disparities a focus of the class (see course syllabus in Appendix). Often the best way to learn is to teach. She is having her students write a final project on a cancer of their choice about the epidemiology of the cancer and to identify an area where a health disparity exists. She is hoping the students will teach the teacher. Taking on this class has given Dr. Torkko invaluable experience in teaching and should lead to other teaching and career development opportunities. It has also been an important review of epidemiology for her as concepts continue to evolve since the time she took basic epidemiology classes.

Readings. Dr. Torkko is currently reading "The Spirit Catches You and You Fall Down" which she plans to discuss with her health disparities mentor, Dr. Angela Sauaia.

**Research Project Progress**

Sociodemographic Survey

*Specific Aim #1: Collect sociodemographic information on SABOR participants using a questionnaire and determine whether sociodemographic factors relating to prostate cancer screening, diagnosis, and treatment differ by race/ethnicity in the SABOR study. Differences in*

*proportions or frequencies of sociodemographic factors will be tested by racial/ethnic group in men with prostate cancer.*

Development and implementation of the survey had been delayed. Currently Dr. Torkko is collecting existing surveys and following up on contacts made during the conference in Atlanta. It is expected that a draft survey will be completed in the next month and sent to UTHSCSA for comment and approval. Their local IRB process will have to be initiated and the survey will be translated into Spanish. The whole process may take four to six months before the survey is ready to be mailed to SABOR participants.

Participation in the AACR Science of Cancer Health Disparities reinforced the importance of taking measures of SES, health status/beliefs, and other cultural issues into account when conducting and analyzing studies.

Single Nucleotide Polymorphisms

*Specific Aim #2: Determine whether VDR polymorphisms, haplotypes, and gene-gene interactions differ by race/ethnicity. Men will be genotyped for VDR, CYP27B1, and CYP24 polymorphisms. A genetic association case-control study will be performed looking for associations of these polymorphisms and haplotypes with prostate cancer.*

The first step of the research plan was to identify a panel of ingle nucleotide polymorphisms (SNPs) for the genes of interest. With the assistance of Dr. Robin Leach at UTHSCSA, a panel of 21 *VDR* SNPs, and 31 SNPs in vitamin D associated genes (*CYP27B1, CYP24A1, PDF*) has been assembled (see Appendix for a list of the SNPs). These SNPs were chosen as tag SNPs to identify known haplotypes in each gene. As part of her research, Dr. Leach is studying genes in the testosterone pathway and has developed an extensive panel of tagSNPs. There will be opportunities for Dr. Torkko to use these SNPs to study gene-gene interactions between vitamin D and testosterone metabolic pathway genes.

Currently, appropriate cases and controls from the SABOR study are being selected and prepared for genotyping. Unfortunately there were delays in this process due to data management issues. The database for the SABOR study had grown to the point the researchers needed to transfer management of the data to the departmental/university IT services. This has resulted in some compatibility problems that have largely been resolved. Hopefully, genotyping will be completed before the middle of 2008. Data analysis will commence as soon as data are available.

*Specific Aim #3: Determine the combined relationships of sociodemographic, clinical, /pathological, and genetic factors to prostate cancer and if these relationships differ by race/ethnicity.*

This aim will need to wait for completion of the previous two aims.

## Key Research Accomplishments

At this point in the grant, the key accomplishments were the publication of two papers in *Clinical Cancer Research* (See Appendix). The first (Shook *et al, Clin Cancer Res* 2007;13:5959-64) was a collaborative effect with researchers at UTHSCSA. The second (Torkko *et al, Clin Cancer Res* 2008 – in press) was on the results from Dr. Torkko's PhD work. Additional genotyping needed to be performed to meet the requirements of the journal reviewers. The grant was acknowledged in the paper as it allowed time and resources to complete the work and get it published.

The main finding of the Shook paper was that in Hispanic White (HW) men and African American men with prostate cancer the odds ratios were 4.4 and 10.4, respectively, for having the *RNASEL* 462 polymorphism AA genotype compared to Non-Hispanic White men (NHW). *RNASEL* had been

previously identified as a hereditary prostate cancer susceptibility gene. This paper was the first to examine this gene in HW men.

The main finding of the Torkko paper was that the vitamin D and testosterone pathways interact to increase risk for prostate cancer in NHW and HW men, and this interaction appears to differ slightly by ethnicity. The *SRD5A2* V89L VV genotype interacts with *VDR Fok*I TT/CT genotypes in NHW men and *VDR* CDX2 GG genotypes in HW men to increase risk for prostate cancer

## Reportable Outcomes

Other than the two papers listed in the section above for the research part of the grant, reportable outcomes that are related to the training activities of the grant and are in the Appendix. These include the proposal written for the Chronic Disease Epidemiology class, the syllabus for the Introductory Epidemiology class, and the transcript for the classes taken so far.

## Conclusions

A substantial amount of work has been done for the first year of the grant, but much work still remains, particularly for the research plan. The training part of the grant has been successful in increasing knowledge and understanding of prostate cancer epidemiology and issues of health disparities in cancer. Additional classes are planned for the fall and spring semesters in the next academic year. The research portion of the grants needs to take priority for the second year to complete the genotyping and to conduct the sociodemographic survey.

## References

Shook SJ, Beuten J, Torkko KC, Johnson-Pais TL, Troyer DA, Thompson IM, Leach RJ. Association of RNASEL variants with prostate cancer risk in Hispanic Caucasians and African Americans. *Clin Cancer Res.* 2007;13:5959-64.

Torkko KC, van Bokhoven A, Mai P, Beuten J, Balic I, Byers TE, Hokanson JE, Norris JM, Baron A, Lucia MS, Thompson IM, and Leach RJ. VDR and SRD5A2 Polymorphisms Combine to Increase Risk for Prostate Cancer in Non-Hispanic White and Hispanic White Men. *Clin Cancer Res* (Accepted for publication)

## Appendices

### A: Torkko *et al. Clin Cancer Res* Paper in Press

*VDR* and *SRD5A2* Polymorphisms Combine to Increase Risk for Prostate Cancer in Non-Hispanic White and Hispanic White Men

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Running title: Interaction between *VDR* and *SRD5A2* in Prostate Cancer

Key words: prostate cancer, vitamin D receptor, *SRD5A2*, genetic association study, haplotypes, interaction

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ABSTRACT

**Purpose**: Vitamin D and dihydrotestosterone pathways interact to promote growth of prostatic tissue. The nuclear Vitamin D receptor (*VDR*) moderates the actions of Vitamin D. 5α-Reductase type II (*SRD5A2*) codes for the enzyme that converts testosterone to dihydrotestosterone in the prostate. This study tested interactions of *VDR* (CDX2, *Fok*I) and *SRD5A2* (V89L, A49T) polymorphisms and their associations with prostate cancer.
**Experimental Design**: This genetic association study included 932 non-Hispanic White (NHW) men and 414 Hispanic White (HW) men from south Texas. Cases had biopsy-confirmed cancer; controls had normal digital rectal exams and serum prostate specific antigen <2.5 ng/ml.
**Results**: Using logistic regression analyses to test associations with prostate cancer, only the V89L polymorphism (VV genotype compared to LL/LV) in HW men was statistically significant (OR=0.64; 95%CI: 0.41, 0.99). The interaction terms for *Fok*I and V89L in NHW men and CDX2 and V89L in HW men in the logistic model were significant (p=0.02 and 0.03, respectively). When stratified by V89L genotype, the *Fok*I polymorphism (TT/TC vs. CC) was significantly associated with prostate cancer in NHW men with the V89L VV genotype (*Fok*I OR=1.53, 95%CI: 1.06, 2.23). The CDX2 polymorphism (GG vs. AG/AA) was significantly associated with prostate cancer only in HW men with the V89L VV genotype (CDX2 OR=3.16, 95%CI: 1.39, 7.19; interaction term p=0.02).
**Conclusion**: Our results indicate that the *SRD5A2* V89L VV genotype interacts with *VDR Fok*I TT/CT genotypes in NHW men and *VDR* CDX2 GG genotypes in HW men to increase risk for prostate cancer.

INTRODUCTION

Prostate cancer is the most commonly diagnosed non-skin cancer and one of the ten leading causes of death in American men.(1) The etiology of prostate cancer is not well known, although both genetic and environmental factors are believed to play a role. A twin study from Scandinavia estimated that 42% of the risk for prostate cancer might be explained by heritable factors.(2) A diverse range of foods and nutrients have been found to moderately affect risk for prostate cancer, including soy, isoflavones, milk, saturated fats, and tomato products.(3)

A link between prostate cancer and vitamin D has been hypothesized. Lower levels of vitamin D in the serum have been associated with increased prostate cancer risk.(4) *In vitro* studies have found that treating prostate cancer cells with vitamin D inhibits cell proliferation.(5) Given these observations, it has been proposed that adequate circulating levels of vitamin D are important to protect against prostate cancer.

The androgen testosterone and its bioactive form, dihydrotestosterone (DHT), are necessary for the normal growth and development of the prostate and epidemiologic evidence supports their role in the etiology of prostate cancer.(6) 5α-reductase type II is the primary enzyme that converts testosterone to DHT in the prostate.(7) Men who lack the gene that codes for 5α-reductase type II have low DHT levels and small prostates.(8) Finasteride, an inhibitor of 5α-reductase type II, reduces the growth of cells from the androgen-dependent LNCaP prostate cancer cell line(9) and is associated with a decrease in tissue DHT levels.(10) The Prostate Cancer Prevention Trial (PCPT) demonstrated that men given finasteride had a 24.8% reduction in cancer prevalence over seven years compared to men given placebo.(11) Increased expression of 5α-reductase type II is also associated with recurrent and metastatic prostate cancer implying a role for the enzyme and DHT in prostate cancer progression.(12)

Growth and differentiation of normal prostatic tissue is promoted by interactions between the vitamin D and DHT pathways.(13) Levels of the bioactive form of vitamin D, calcitriol, are controlled in an autocrine fashion to regulate cell growth and decrease the risk of the cells becoming malignant. DHT appears to act as a regulator of vitamin D activity. When cells from the prostate cancer cell line LNCaP are grown in androgen-depleted medium, vitamin D no longer inhibits cell growth. With the addition of

DHT, even at low physiologic levels (1 nM), the anti-proliferative effects of vitamin D are restored.(14) It was later demonstrated that this effect is mediated by DHT-induced suppression of 24-hydroxylase expression, the enzyme that inactivates calcitriol(15) and its precursor form.(14) Additionally, in two androgen receptor(AR)-positive prostate cancer cell lines (DHT binds to AR), AR signaling was shown to be required for the vitamin D-mediated growth inhibition of the cancer cells.(16) This sets up a paradox of androgens being associated with higher risk for cancer development, but at the same time being important for the anti-cancer activities of vitamin D.

Located on chromosome 12q13-q14, the high-affinity nuclear vitamin D receptor gene (*VDR*) mediates most of the biological activity of vitamin D.(17) If vitamin D can regulate the growth of normal and cancerous prostate cells, then variations in the activity of the *VDR* may be important in the onset and progression of prostate cancer. Two of the commonly studied *VDR* polymorphisms, *Fok*I and CDX2, result in functional changes. The *Fok*I (T/C) variant alters the translation start site resulting in two isoforms of the VDR protein with differing activities(18), with the protein product from the *Fok*I T form exhibiting less transcriptional activation than the product from the wild-type C form.(19) The presence of the *Fok*I C allele was found to affect immune cell behavior resulting in a more active immune system.(20) The CDX2 variant in the promoter region of the *VDR* modulates promoter activity, and the CDX2 G allele, the most common allele, shows 30% less transcriptional activity compared to the A allele.(21) Several studies of the *Fok*I polymorphism and its association with prostate cancer have produced inconsistent results and a meta-analysis of several *VDR* polymorphisms concluded that *Fok*I was unlikely to have a major role in prostate cancer.(22) CDX2 has been less extensively studied but it was found to increase risk for prostate cancer in men with the heterozygous genotype and high ultraviolet B exposure.(23)

The gene that codes for 5α-reductase type II, *SRD5A2* located on chromosome 2, has several polymorphisms that have been studied for their relationship with prostate cancer. The most common polymorphism is V89L that substitutes valine at codon 89 with leucine by a C to G nucleotide transversion. The leucine allele (L) reduces 5α-reductase activity resulting in lower DHT levels.(24,25). The A49T polymorphism results in a threonine substitution for alanine and is associated with increased 5α-reductase activity *in vitro* causing increased DHT production that may contribute to prostate cancer development or progression.(26) The relationship of the V89L and A49T polymorphisms with prostate cancer has not been proven conclusively. A meta-analysis of *SRD5A2* polymorphisms concluded that the V89L polymorphism likely has no, or little, relationship to prostate cancer risk and that A49T may have a modest effect accounting for only a small proportion of prostate cancer.(27)

Because of the complex etiology of prostate cancer, the effects of many individual genetic polymorphisms are likely to be small. It is possible that larger effects may only be observed when polymorphisms are considered in combination. A polygenic model incorporating multiple loci might maximize detection of individuals at high-risk for prostate cancer.(28)

The current study tested possible interactions of the *VDR* and *SRD5A2* genes as identified by two functional polymorphisms in each gene in determining risk for prostate cancer in a cohort of Non-Hispanic White and Hispanic White men from south Texas. The ***a priori*** hypotheses of this study were that the *Fok*I T allele and the CDX2 G allele, that both result in decreased vitamin D receptor activity, in combination with the V89L V or A49T T alleles, that result in higher levels of DHT, would lead to increased risk for prostate cancer. Although DHT is important for vitamin D activity and higher DHT levels might be hypothesized to reduce risk by increasing vitamin D levels, we believe that the less efficient vitamin D receptor as indicated by the presence of the *Fok*I T and CDX2 G alleles will not utilize the higher vitamin D levels to counter the increased risk posed by higher DHT levels.

MATERIALS AND METHODS

Study Population. Study participants came from the population-based prospective SABOR cohort study (San Antonio Biomarkers Of Risk for prostate cancer) at the University of Texas Health Sciences Center, San Antonio (UTHSCSA).(29) SABOR began enrolling men in May 2001 to examine differences in risk for prostate cancer by race/ethnicity. Three racial/ethnic groups reflecting the diversity of the southern Texas population were enrolled: non-Hispanic Whites (NHW), Hispanic Whites (HW), and African Americans. Only NHW and HW men were used in this study due to limited numbers of African American men (less than 65 prostate cancer cases). Race is self-identified and Hispanic ethnicity was assigned using the Hazuda model for the identification of Mexican Americans and other Hispanic ethnicities.(30) The Hispanic population of south Texas is approximately 95% Mexican American. All participants were consented for the genetic studies according to UTHSCSA Institutional Review Board's rules and regulations.

Cases in this analysis were men with histologically-confirmed prostate cancer in the SABOR cohort, as well as men diagnosed with confirmed prostate cancer from the same clinics and health fairs from which the SABOR cohort was recruited. Gleason scores (range 2-10) were determined from chart reviews. High-grade cancers were defined as cases with Gleason scores of seven or greater. Prostatectomy scoring was used preferentially over biopsy scores when available.

Controls, selected from the SABOR cohort, were eligible for this analysis if they had prostate-specific antigen (PSA) values less than 2.5 ng/ml at all visits (up to five annual visits) and a normal digital rectal exam (DRE) at all visits. Age, defined as age at diagnosis for the cases and age at last visit for the controls, was truncated at 45 years old and above for both cases and controls. The study population consisted of 1,346 men for a total of 585 cases and 761 controls. HW men accounted for 44% of the study sample.

Polymorphism Selection and Genotyping. Two *VDR* polymorphisms and two *SRD5A2* polymorphism were genotyped: CDX2 (rs17883968; G/A) in the *VDR* promoter region, *Fok*I (rs10735810; C/T) in *VDR* exon 2, and V89L (rs523349) and A49T (rs9282858) in exon 1 of the *SRD5A2* gene.

DNA for genotyping was extracted from blood samples using a QIAamp blood kit (QIAGEN, Valencia, CA). Genotyping for CDX2, V89L, and A49T was performed with TaqMan allelic discrimination assays using the ABI 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). Originally a TaqMan assay could not be successfully designed for *Fok*I. This polymorphism was genotyped using endonuclease restriction enzyme digestion. Subsequently, a *Fok*I kit was developed and purchased. To do a quality control check on the original *Fok*I genotyping, 324 men (19% of the sample) were re-genotyped using the TaqMan kit. There was only one discrepancy between the two methodologies for an error rate of 0.3%. Applied to our larger sample of 1,685 men this means there are potentially 5 men who are discordant. We feel this is an acceptable error rate and that the original methodology is validated. All genotyping was performed in a molecular genetics laboratory at UTHSCSA.

Men homozygous for each risk allele in the individual polymorphisms were compared to heterozygotes and homozygotes for the complimentary allele combined. Men homozygous for the *VDR* CDX2 risk allele (G) were compared to men with AG or AA genotypes. For the *SRD5A2* V89L polymorphism, the VV genotype was compared to LL and LV genotypes in all analyses. Due to a limited number of men homozygous for the risk alleles in the *VDR Fok*I and *SRD5A2* A49T polymorphisms, the risk genotype was combined with the heterozygous genotype and compared to men homozygous for the complementary allele. Thus for *Fok*I the comparison was between TT/CT and CC genotypes and for A49T it was between the TT/AT and AA genotypes if any TT genotypes are found.

Statistical Analyses. All analyses were stratified by ethnicity. Associations between genotypes and prostate cancer were assessed by chi-square tests (Pearson Chi-square with one or two degrees of

freedom) and logistic regression analyses. All logistic regression models included age as a continuous variable. Interactions between *VDR* and *SRD5A* polymorphisms were tested in the logistic regression analyses by adding an interaction term to the model. Nominal logistic regression was used to test the relationship of the Gleason score groups (low grade 2-6 and high grade 7-10) to controls as the referent group. Alpha levels of 0.05 were used for hypothesis testing, and 95% confidence intervals were computed for all relative risk estimates (odds ratios). For NHW men, the study sample size had 80% power (alpha=0.05) to detect at least a 25% difference in proportions of genotypes between cases and controls based on published reports of genotype proportions in controls. For HW men, the detectable difference was 35%. Analyses were completed using SAS 9.1 statistical software (SAS institute, Inc., Cary, NC).

## RESULTS

The study sample consisted of 932 NHW men (444 cases, 488 controls) and 414 HW men (141 cases, 273 controls; Table 1). Controls were somewhat younger than cases in both ethnic groups. Gleason score distribution was not different between ethnic groups.

Genotype distributions for the individual polymorphisms within each ethnic group did not differ by case-control status (Table 2). Genotype distributions for controls differed by ethnicity, however, for the *VDR Fok*I and the *SRD5A2* V89L polymorphisms. About 13% of NHW controls had the *Fok*I TT genotype compared to 21% of HW controls (p=0.009). For the V89L polymorphism, 52% and 44% of NHW and HW controls, respectively, had the VV genotype (p=0.001). The genotype distributions in controls for these polymorphisms do not differ significantly from previously published results.(31,32) Additionally, CDX2 genotype distributions in NHW controls are similar to what was found earlier.(33) There are no published data on CDX2 for HW men.

All polymorphisms were in Hardy-Weinberg equilibrium within each ethnic group. Odds ratios and 95% confidence intervals for the hypothesized risk genotypes are presented in Table 2. The *SRD5A2* A49T AT genotype was compared to the AA genotype as there were no homozygous TT genotypes in the sample. Only the V89L polymorphism in HW men was marginally significant (VV OR=0.64; 95%CI: 0.41, 0.99; p=0.05). No significant results were seen with the A49T polymorphism and, given the small numbers of men with the T allele, no interaction analyses were performed with this polymorphism.

Evidence of effect modification of the VDR *Fok*I polymorphism by *SRD5A* V89L was found (logistic regression interaction term p=0.02). When the effect of the *Fok*I polymorphism was analyzed by V89L genotype, the previously non-significant *Fok*I effect was significant in NHW men (Table 3). In men with the V89L VV genotype, men with the *FokI* TT or CT genotypes were at a 50% increased risk for prostate cancer (OR=1.53; 95%CI: 1.06, 2.23; p=0.03). There was no evidence of interaction between *Fok*I and V89L in HW men.

There was evidence of effect modification of the *VDR* CDX2 polymorphism by V89L in HW men (logistic regression interaction term p=0.03). Men with the higher-risk V89L VV genotype combined with another higher-risk genotype, the CDX2 GG genotype, to increase risk for prostate cancer. HW men with the CDX2 GG and V89L VV genotypes have more than three times the risk for prostate cancer (CDX2 GG OR=3.16; 95%CI: 1.39, 7.19; p=0.01; Table 4). There was no evidence of interaction in NHW men.

The individual polymorphisms were investigated for their associations with higher Gleason score, the measure of cancer grade. Gleason score is an important predictor of disease progression.(34) Decrease in differentiation as measured by the Gleason grade is related to lack of tissue function and the Gleason score correlates with overall disease–free survival: the higher the score, the more likely that disease will recur.(35) There was no evidence of associations with Gleason grade in HW men or in NHW men (results not shown).

## DISCUSSION

This study is one of the few to examine genetic risks for prostate cancer in a group of Hispanic men. Using a population of Non-Hispanic White men and Hispanic White (largely Mexican American) men from South Texas, we found evidence of interaction between three functional polymorphisms from two genes in the vitamin D and androgen pathways to affect risk for prostate cancer. In NHW men there was an interaction between the *VDR Fok*I and *SRD5A2* V89L polymorphisms to increase risk in men with the *Fok*I TT or CT genotypes and the V89L VV genotype. On the other hand, in HW men, the interaction for increased risk was between the *VDR* CDX2 GG and V89L VV genotypes.

This study examined two genes potentially involved with prostate cancer risk in combination. A polygenic approach may be a more appropriate method to study genetic associations with complex diseases such as cancer.(28) The association of *Fok*I with colon cancer was seen only when analyzed in women with <23 CAG repeats in the androgen receptor.(36) The association with prostate cancer aggressiveness of a polymorphism in a gene that codes for an enzyme involved with the degradation of DHT, 3 beta-hydroxysteroid dehydrogenase type II, is strengthened when analyzed by *SRD5A* V89L genotype.(37)

This study found heterogeneity of effects by ethnicity. Neither *Fok*I nor V89L alone was associated with prostate cancer in NHW men, but taken together, the odds for disease are increased 50% in men with the *Fok*I TT/CT and V89L VV genotypes. No such association was found in HW men. HW men had more than three times the odds of prostate cancer if they had the CDX2 GG and the V89L VV genotypes. Previous studies have also observed heterogeneity of effects by ethnicity with the *Fok*I polymorphism. For example, a significant trend for increasing waist-to-hip ratio with *Fok*I genotype was found in Hispanic women but not in NHW women.(31)

Differences in linkage disequilibrium to unmeasured genes and/or gene-gene interactions may contribute to the differences found by ethnicity. It is possible that these differences may depend on the different combinations of these genes, or other unmeasured genes, either linked or unlinked to the *Fok*I, CDX2, and V89L polymorphisms. The findings of this study suggest that associations and interactions of the *VDR* and *SRD5A* polymorphisms may be specific to ethnicity, arguing that research results should be stratified by race or ethnicity.

The association of the *SRD5A* V89L polymorphism with prostate cancer ran counter to our hypothesized effect. We hypothesized that the VV genotype would be associated with increased risk for prostate cancer compared to the LL genotype because the L allele is associated with a moderate reduction in 5α-reductase type II activity resulting in lower DHT levels.(24) A meta-analysis of *SRD5A2* polymorphisms, however, concluded that the V89L polymorphism likely has no, or little, relationship to prostate cancer risk.(27) Most of the studies in the meta-analysis were done in NHW or African American men. Information on Hispanic men is sparse. A 2005 study in Southern California found that Hispanics with the LL genotype were at significantly increased risk from prostate cancer compared to men with the VV genotype (OR=7.3, 95%CI: 1.5,35.5), although this finding is based on only 84 cases and 44 controls of which only 2 controls had the LL genotype.(38) In the current study, HW men with the *SRD5A* V89L VV genotype had a *reduced* risk compared to the VL/LL genotypes (OR=0.64; 95%CI: 0.41, 0.99; p=0.05). There was no association with risk in NHW men. The result in HW men was marginal, however, and may reflect a more limited sample size in HW men. These findings need to be studied in a larger cohort.

In contrast to associations with prostate cancer risk, several studies found that the LL genotype was associated with increased risk for measures of disease *severity* or *progression*.(37) For example, the LL genotype was associated more aggressive disease(39), a poorer prognosis as measured by PSA failure,(40) and by the presence of metastases at the time of diagnosis.(41) Thus it appears that reduced DHT is associated with increased risk for disease *progression*.(42)

HW men in this study have a higher proportion of the LL genotype (15%) than NHW men (7%). Thus it appears that HW men are more likely to have a less efficient *SRD5A2* gene and therefore less DHT available. This could partly explain the paradox that overall HW men have lower prostate cancer rates but are more likely to have higher clinical stage at diagnosis(43), poorer survival(44), and more non-localized disease(45) than NHW men. A recent study looked at the distribution of V89L polymorphisms

in low-risk Inuit natives in Greenland compared to high-risk Swedish men. The proportion of the higher activity VV V89L genotype was significantly lower in Inuits compared to Swedish men.(46) The authors hypothesized this contributes to the lower of risk prostate cancer seen in the Inuit.

The cases in the SABOR study are largely prevalent rather than incident cases. Most men who were diagnosed during the up to five annual SABOR exams probably had already developed the disease that only became clinically evident during the increased surveillance as part of their participation in the study. Therefore, it is difficult to discern between markers that are associated with initiation or with progression of the disease. Long-term follow-up is needed to determine which cancer cases will progress. Although Gleason score is an imperfect measure of cancer progression, it can be useful to determine between the high-risk (usually Gleason score 7 and above) versus lower-risk cases. Even though no overall association with Gleason score was observed, the high-risk HW cases were more likely to have the V89L LL genotype (23%) than the low-risk cases (9%); there is no difference in NHW men (6% and 7%, respectively).

The presence of population stratification (genetic subgroups), particularly in HW men, could lead to inaccurate estimates of the genetic effects if the subgroups are not equally distributed between cases and controls. A recent study comparing admixture and substructure in Mexicans and Puerto Ricans, the two largest Hispanic/Latino subgroups in the US, found population substructure in both groups.(47) However, in their study of asthma, they found this substructure only confounded their results in Puerto Ricans and not Mexicans. The effect of population stratification may be important only if the substructure includes populations that have differential risk for the disease of interest and differential distributions of the gene of interest.(48) Mexican Americans, who comprise >90% of the SABOR sample, are primarily made up of European and Native American ancestries. Native Americans are at lower risk for prostate cancer compared to NHW men.(49) Only one of the polymorphisms in the current study has been examined in a native population, the Inuits in Greenland, where the proportion of the higher activity V89L VV genotype was significantly lower in Inuits compared to Europeans.(46) Depending on the percentage of native admixture in the SABOR Hispanic population and if there are different distributions between cases and controls, there could be an inaccurate estimate of the risk effect for the V89L polymorphism or the other polymorphisms in this study. Although a source of systematic bias has not been identified, a panel of ancestry informative markers on the SABOR population is being run to study this issue.

This study found evidence that the *SRD5A2* V89L polymorphism interacts with the functional *VDR Fok*I and CDX2 polymorphisms to affect risk for prostate cancer in Non-Hispanic and Hispanic White men, respectively. This illustrates the importance of examining multiple genes to understand genetic risks for prostate cancer and differences seen by ethnicity. Additionally, a complex analysis may be necessary to understand a complex disease. Because genome-wide linkage studies found strong locus heterogeneity of prostate cancer susceptibility genes(50), prostate cancer is not likely caused by a few genes but by multiple genes from different pathways. Therefore, a more complex analysis looking at interactions between genes rather than a single gene analysis may be necessary to understand complex diseases like prostate cancer.

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**Table 1.** Participant characteristics

| | | Non-Hispanic White | | Hispanic White | |
|---|---|---|---|---|---|
| | | **Cases** | **Controls** | **Cases** | **Controls** |
| N | | 444 | 488 | 141 | 273 |
| Age (years) | 45-59 | 94 (21%) | 182 (37%) | 43 (31%) | 157 (57%) |
| | 60-69 | 203 (46%) | 185 (38%) | 60 (42%) | 95 (35%) |
| | 70+ | 147 (33%) | 121 (25%) | 38 (27%) | 21 ( 8%) |
| | Mean | 66.5 | 64.1* | 64.4 | 59.2* |
| Gleason Score | 2-5 | 49 (15%) | | 10 ( 9%) | |
| | 6 | 147 (44%) | | 54 (47%) | |
| | 7 | 90 (27%) | | 33 (28%) | |
| | 8-10 | 46 (14%) | | 19 (16%) | |
| | Median | 6 | | 6** | |

*p<0.0001 for differences between cases and controls in both ethnic groups (T-test)
**p=0.23 for differences between NHW and HW men (Wilcoxon Rank Sum test)

**Table 2.** Distribution of *VDR Fok*I and CDX2 and *SRD5A2* V89L and A49T polymorphisms by case-control status in Non-Hispanic White (NHW) cases (n=439) and controls (n=488), and in Hispanic White (HW) cases (n=140) and controls (273).

| Poly-morphism | Ethnicity | Geno-type | Number (%) Cases | Number (%) Controls | Chi-square p value† | Genotype Comparison | OR (95% CI) |
|---|---|---|---|---|---|---|---|
| *VDR* CDX2 | NHW | GG | 282 (64) | 323 (66) | 0.05 | GG vs. | 0.87 (0.67, 1.15) |
| | | AG | 131 (29) | 148 (30) | | AG/AA (ref) | |
| | | AA | 31 ( 7) | 17 ( 3) | | | |
| | HW | GG | 98 (69) | 174 (64) | 0.32 | GG vs. | 1.57 (0.99, 2.50) |
| | | AG | 38 (27) | 81 (30) | | AG/AA (ref) | |
| | | AA | 5 ( 4) | 18 ( 7) | | | |
| *VDR Fok*I* | NHW | TT | 67 (15) | 63 (13) | 0.54 | TT/CT | 1.12 (0.86, 1.46) |
| | | CT | 209 (47) | 227 (46) | | vs. CC (ref) | |
| | | CC | 168 (38) | 198 (41) | | | |
| | HW | TT | 26 (18) | 57 (21) | 0.73 | TT/CT | 1.00 (0.68, 1.57) |
| | | CT | 70 (50) | 125 (46) | | vs. CC (ref) | |
| | | CC | 45 (32) | 91 (33) | | | |
| *SRD5A2* V89L* | NHW | VV | 230 (52) | 251 (52) | 0.93 | VV vs. | 1.06 (0.82, 1.38) |
| | | LV | 185 (42) | 202 (41) | | LV/LL (ref) | |
| | | LL | 29 ( 6) | 35 ( 7) | | | |
| | HW | VV | 52 (37) | 119 (44) | 0.24 | VV vs. | 0.64 (0.41, 0.99) |
| | | LV | 70 (50) | 112 (41) | | LV/LL (ref) | |
| | | LL | 19 (13) | 42 (15) | | | |
| *SRD5A2* A49T | NHW | TT | 0 ( 0) | 0 ( 0) | 0.97 | AT vs. | 1.06 (0.65, 1.75) |
| | | AT | 33 ( 7) | 36 ( 7) | | AA (ref) | |
| | | AA | 411 (93) | 452 (93) | | | |
| | HW | TT | 0 ( 0) | 0 ( 0) | 0.94 | AT vs. | 1.32 (0.46, 3.73) |
| | | AT | 6 ( 4) | 12 ( 4) | | AA (ref) | |
| | | AA | 135 (96) | 261 (96) | | | |

*Significant differences in genotype distributions in controls between NHW and HW men (p=0.009 for FokI; p=0.001 for V89L).

† Pearson Chi-square test with 2 d.f.

**Table 3.** Distribution of *VDR Fok*I genotypes stratified by *SRD5A2* V89L LL/LV and VV genotype groups with age-adjusted logistic regression odds ratios (OR) and 95% confidence intervals (95%CI) for associations of *Fok*I TT/CT genotypes with prostate cancer in Non-Hispanic White (NHW) and Hispanic White (HW) men.

| Ethnicity | *V89L* Genotype | *Fok*I Genotype | Number (%) Cases | Number (%) Controls | Chi-square p value† | *Fok*I OR (95%CI) | p-value |
|---|---|---|---|---|---|---|---|
| NHW* | All | TT/CT | 276 (62) | 290 (59) | 0.39 | 1.12 (0.86,1.46) | 0.41 |
| | | CC | 168 (38) | 198 (41) | | 1.0 | |
| | VV | TT/CT | 152 (66) | 142 (57) | 0.03 | 1.53 (1.06,2.23) | 0.03 |
| | | CC | 78 (34) | 109 (43) | | 1.0 | |
| | LV/LL | TT/CT | 124 (58) | 148 (62) | 0.33 | 0.79 (0.54, 1.16) | 0.23 |
| | | CC | 90 (42) | 89 (38) | | 1.0 | |
| HW** | All | TT/CT | 96 (68) | 182 (67) | 0.77 | 1.00 (0.63, 1.57) | 0.99 |
| | | CC | 45 (32) | 91 (33) | | 1.0 | |
| | VV | TT/CT | 40 (77) | 83 (70) | 0.34 | 1.43 (0.66, 3.13) | 0.36 |
| | | CC | 12 (23) | 36 (30) | | 1.0 | |
| | LV/LL | TT/CT | 56 (63) | 99 (64) | 0.83 | 0.86 (0.49, 1.54) | 0.62 |
| | | CC | 33 (37) | 55 (36) | | 1.0 | |

*Interaction term in full logistic regression model for FokI-V89L p=0.02
**Interaction term in full logistic regression model for FokI-V89L p=0.32
† Pearson Chi-square with 1 d.f.

**Table 4.** Distribution of *VDR* CDX2 genotypes stratified by *SRD5A2* V89L LL/LV and VV genotype groups with age-adjusted logistic regression odds ratios (OR) and 95% confidence intervals (95%CI) for associations of CDX2 GG genotype with prostate cancer in Non-Hispanic White (NHW) and Hispanic White (HW) men.

| Ethnicity | *V89L* Genotype | CDX2 Genotype | Number (%) Cases | Number (%) Controls | Chi-square p value† | CDX2 OR (95%CI) | p-value |
|---|---|---|---|---|---|---|---|
| NHW* | All | GG | 282 (64) | 323 (66) | 0.39 | 0.87 (0.67, 1.14) | 0.34 |
| | | AG/AA | 162 (36) | 165 (34) | | 1.0 | |
| | VV | GG | 140 (61) | 164 (65) | 0.31 | 0.82 (0.57, 1.20) | 0.31 |
| | | AG/AA | 90 (39) | 87 (35) | | 1.0 | |
| | LV/LL | GG | 142 (66) | 159 (67) | 0.87 | 0.93 (0.63, 1.39) | 0.74 |
| | | AG/AA | 72 (34) | 78 (33) | | 1.0 | |
| HW** | All | GG | 98 (69) | 174 (64) | 0.24 | 1.57 (0.99, 2.50) | 0.05 |
| | | AG/AA | 43 (31) | 99 (36) | | 1.0 | |
| | VV | GG | 42 (81) | 74 (62) | 0.02 | 3.16 (1.39, 7.19) | 0.01 |
| | | AG/AA | 10 (19) | 45 (38) | | 1.0 | |
| | LV/LL | GG | 56 (63) | 100 (65) | 0.75 | 1.13 (0.63, 2.02) | 0.68 |
| | | AG/AA | 33 (37) | 54 (35) | | 1.0 | |

*Interaction term in full logistic regression model for CDX2-V89L p=0.63
**Interaction term in full logistic regression model for CDX2-V89L p=0.03
† Pearson Chi-square with 1 d.f.

## Appendices – cont.

### B: Shook *et al Clin Cancer Res* paper

# Association of RNASEL Variants with Prostate Cancer Risk in Hispanic Caucasians and African Americans

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**Abstract**

**Purpose:** The *RNASEL* gene at 1q25 has been identified as a hereditary prostate cancer susceptibility gene, but to date, no study has investigated the role of RNASEL variants in Hispanic Caucasian men with prostate cancer.

**Experimental Design:** Two RNASEL common variants, located at amino acids 462 and 541, were genotyped in non-Hispanic Caucasian, Hispanic Caucasian, and African American prostate cancer cases and controls.

**Results:** The RNASEL 462 AA genotype was found to increase prostate cancer risk over 4-fold in Hispanic Caucasians [odds ratio (OR), 4.43; 95% confidence interval (95% CI), 1.68-11.68; $P = 0.003$] and over 10-fold in African Americans (OR, 10.41; 95% CI, 2.62-41.40; $P = 0.001$) when compared with the GG genotype. Analysis of the RNASEL 541 variant showed that Hispanic Caucasian patients with the GG genotype had a statistically significant increase in their risk for developing prostate cancer when compared with the TT and GT genotypes (OR, 1.91; 95% CI, 1.16-3.14; $P = 0.01$). A common G-T haplotype for the combination of the RNASEL 462 and 541 variants was found to occur more frequently in controls compared with cases in African Americans ($P = 0.04$) but not in non-Hispanic Caucasians or Hispanic Caucasians.

**Conclusions:** This is the first study that investigates the association of prostate cancer risk with RNASEL variants in Hispanic men. Our data support the role of RNASEL as a predisposition gene for prostate cancer and showed a significant association between the RNASEL 462 variant and prostate cancer risk in African Americans and Hispanic Caucasians.

Over 218,000 men in the United States are estimated to be diagnosed with prostate cancer (MIM 176807) in 2007 alone and approximately 27,000 men will die from it (1). Although prostate cancer is the most common non-skin cancer and the second leading cause of cancer death in men in the United States, little is known about inherited factors that influence its genetic predisposition. Many factors are known to contribute to the risk of prostate cancer, including diet, race and ethnicity, age, sexual history, and family history (2–6).

Currently, elevated serum levels of prostate-specific antigen and/or an abnormal digital rectal exam are the main methods for diagnosing this disease (7). However, there is increased impetus for better understanding of the molecular processes involved in prostate carcinogenesis with the ultimate goal of discovering new biomarkers, which may be beneficial in the detection, prevention, and/or treatment of this disease (8). Only limited association studies on candidate genes and/or linkage analyses for susceptibility loci have consistently produced positive findings. In 1996, the first prostate cancer susceptibility locus, the *hereditary prostate cancer* (*HPC*) *1* locus (*HPC1*; MIM 601518), was mapped to chromosomal region 1q24-q25 by linkage analysis (9) and since this initial report, several prostate cancer susceptibility loci have been identified (10–18). Because the majority of these regions have not been consistently confirmed in independent populations, evidence has emerged that prostate cancer is a genetically complex and heterogeneous disorder, with multiple genetic and environmental factors contributing to the disease.

There is substantial evidence for a genetic component in the vulnerability to prostate cancer. A cohort study of twins reported by Lichtenstein et al. (19) indicated that the proportion of prostate cancer risk accounted for by heritable factors is estimated to be 42%. Prostate cancer is classified as hereditary (HPC) or sporadic and it is assumed that HPC might be caused by rare, highly penetrant alleles at single gene forms of the disease (20). Alternatively, the sporadic prostate cancer cases may involve some of the same genes and pathways that determine HPC incidence, but they most likely involve more common, low- to moderate-penetrant alleles in genes that are components of pathways that influence prostate function (21–23).

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An important gene involved in innate immunity and apoptosis is the gene encoding 2′-5′-oligoadenylate (2-5A)–dependent RNASEL (*RNASEL*; MIM 180435). *RNASEL*, located at 1q25, regulates cell proliferation and apoptosis through the IFN-regulated 2-5A pathway (24) that mediates antiviral and antiproliferative activities (25–27) and has been suggested to be a candidate tumor suppressor gene. Previous studies indicated that germ-line mutations in the *RNASEL* gene segregate in prostate cancer families that show linkage to the HPC1 region (28). The investigators also found a truncating mutation (E265X) and an initiation-codon mutation (M1I) segregating with the disease in two HPC1-linked families. Functional studies show that both mutations were associated with a reduction in RNASEL activity (28). Furthermore, loss of the wild-type *RNASEL* allele was found in tumor tissue from an affected patient in a family with the E265X mutation, accompanied by absent protein expression. This E265X mutation was also associated with HPC in Finnish patients (29). Follow-up studies revealed a frameshift mutation, 471delAAAG, as a founder allele in Ashkenazi Jews (30).

There are numerous nucleotide variants identified in the *RNASEL* gene, with seven of them resulting in protein sequence changes (29). Six variants cause missense alterations and one rare variant creates a nonsense mutation. The two most commonly found variants in the U.S. non-Hispanic Caucasian population are the nonsynonymous variants: $Arg^{462}Gln$ (G→A) and $Asp^{541}Glu$ (T→G). The $Arg^{462}Gln$ variant reduces the ability of the cell to cause apoptosis in response to activation by 2-5 (A) and also has three times less enzymatic activity than normal (31), whereas the $Asp^{541}Glu$ variant has no known effect on RNASEL protein function (32). There continues to be much debate over whether these common variants increase the risk of prostate cancer. The $Arg^{462}Gln$ AA genotype has been associated with both increased prostate cancer in U.S. Caucasian sample groups (31, 32) and decreased prostate cancer risk in Caucasian and Japanese sample groups (33, 34). Previous studies using the $Asp^{541}Glu$ variant within RNASEL indicated that the GG and TT genotypes were associated with an increased risk for prostate cancer in Japanese (34) and European-American samples, respectively (17). On the other hand, a significant negative association of the TT genotype with prostate cancer in Swedish Caucasian samples was reported by Wiklund et al. (35).

In summary, several studies provide strong support, both functional and epidemiologic, that RNASEL plays a role in HPC, yet other studies have suggested that its role may be small. To date, no association study has been done using Hispanic Caucasian prostate cancer cases. Furthermore, no significant association has been reported in African American prostate cancer cases thus far. In this study, we analyzed an extended group of samples from three different racial/ethnic groups to determine whether a significant association exists between the allelic variants RNASEL 462 and/or RNASEL 541 and prostate cancer in non-Hispanic Caucasians, Hispanic Caucasians, and/or African Americans.

## Materials and Methods

***Study participants.*** The San Antonio Center for Biomarkers of Risk of Prostate Cancer cohort was used for the study. The San Antonio Center for Biomarkers of Risk of Prostate Cancer is funded by the National Cancer Institute and has been prospectively enrolling healthy male volunteers for over 6 years. Digital rectal exams were done and serum prostate-specific antigen levels were determined at every annual visit. Cases were individuals with a known history of prostate cancer enrolled in a parallel study of prevalent prostate cancer or individuals enrolled in the San Antonio Center for Biomarkers of Risk of Prostate Cancer Study who were diagnosed with prostate cancer. Cases had biopsy-confirmed prostate cancer and controls consisted of male volunteers of at least 45 years old who had normal digital rectal exams and prostate-specific antigen levels of <2.5 ng/mL on at least two and up to six study visits. Race/ethnicity was self-reported. Table 1 shows the characteristics of the study samples. For this study, we used 933 non-Hispanic Caucasians (430 cases and 503 controls), 392 Hispanic Caucasians (150 cases and 242 controls), and 214 African Americans (68 cases and 146 controls). This study received Institutional Review Board approval from the University of Texas Health Science Center at San Antonio. Informed consent was obtained from all subjects.

***DNA isolation and genotyping.*** DNA was isolated from participants' whole blood cells using a QIAamp DNA Blood Maxi kit (Qiagen) and was used for genotyping. The Taqman allelic discrimination assay (Applied Biosystems) was used to genotype the nucleotide variants RNASEL $Arg^{462}Gln$ (rs486907) and $Asp^{541}Glu$ (rs627928). Primers and probes were designed using Primer Express (Applied Biosystems). The primers and probes for $Arg^{462}Gln$ were as follows: forward primer 5′-GGAAGATGTGGAAAATGAGGAAGA-3′, reverse primer 5′-TGCAGATCCTGGTGGGTGTA-3′, and probes 5′-VICCAGGACATTTCGGGCAA-MGB and 5′-FAMCAGGACATTTTGGGCAA-MGB. The primers and probes for $Asp^{541}Glu$ were as follows: forward primer 5′-TCTATGTGGTAAAGAAGGGAAGCA-3′, reverse primer 5′-TTGAACCACCTCTTCATTACTTTGAG-3′, and probes 5′-VICTTTCAGATCCTCAAAT-MGB and 5′-FAMTTTCAGCTCCTCAAAT-MGB. The target sequences were amplified by PCR in 7 μL reaction mix containing

**Table 1.** Characteristics of subjects for this study

| Subgroup | Cases (*n* = 732) | Controls (*n* = 1,546) |
|---|---|---|
| | *n* (%) | *n* (%) |
| Ethnic background | | |
| Non-Hispanic Caucasian | 503 (68.7) | 840 (54.3) |
| Hispanic Caucasian | 159 (21.7) | 501 (32.4) |
| African American | 70 (9.6) | 205 (13.3) |
| Age (y) | | |
| ≤50 | 21 (2.9) | 214 (13.8) |
| 51-60 | 165 (22.5) | 601 (38.9) |
| 61-70 | 329 (45.0) | 485 (31.4) |
| >70 | 217 (29.6) | 246 (15.9) |
| PSA (ng/mL) | | |
| ≤4.0 | 138 | 1,546 |
| 4.1-10.0 | 30 | 0 |
| 10.1-20.0 | 2 | 0 |
| >20.0 | 4 | 0 |
| Mean (SD) | 3.284 (4.21) | 0.895 (0.461) |
| DRE | | |
| Normal | 47 | 1,546 |
| Abnormal | 113 | 0 |
| Family history of PCa | | |
| Negative | 531 (72.5) | 1,260 (81.5) |
| Positive | 201 (27.5) | 286 (18.5) |
| Gleason score | *n* = 560 | |
| <7 | 326 (58.2) | |
| 7 | 153 (27.3) | |
| >7 | 81 (14.5) | |

Abbreviations: PSA, prostate-specific antigen; DRE, digital rectal exam; PCa, prostate cancer; SD, standard deviation.

**Table 2.** Allele frequencies for the more common allele by race/ethnicity and case-control status

| SNP | NCBI reported CEU | NCBI reported YRI | Non-Hispanic Caucasians | | | Hispanic Caucasians | | | African Americans | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cases ($n$ = 430) | Controls ($n$ = 503) | $P$ | Cases ($n$ = 150) | Controls ($n$ = 242) | $P$ | Cases ($n$ = 68) | Controls ($n$ = 146) | $P$ |
| RNASEL 462 G | 0.592 | 0.942 | 0.649 | 0.663 | 0.90 | 0.687 | 0.766 | **0.01** | 0.754 | 0.874 | **0.0005** |
| RNASEL 541 G | 0.625 | 0.217 | 0.545 | 0.560 | 0.66 | | | | | | |
| RNASEL 541 T | | | | | | 0.493 | 0.529 | 0.33 | 0.657 | 0.689 | 0.52 |

Abbreviations: NCBI, National Center for Biotechnology Information; CEU, CEPH (Utah residents with ancestry from northern and western Europe); YRI, Yoruba in Ibadan, Nigeria.

10 ng of genomic DNA, 900 nmol/L of each primer, 200 nmol/L of each probe, and 1× Taqman Universal PCR Master Mix (Applied Biosystems). PCRs were incubated at 95°C for 10 min followed by 40 cycles of denaturing at 95°C for 15 s and annealing/extending at 60°C for 1 min. Genotypes were determined using an ABI 7900HT Sequence Detection System (Applied Biosystems) and analyzed with the SDS 2.0 software (Applied Biosystems). To ensure the quality of the genotyping, consistent results were required for eight control samples added to each 384-well reaction plate. We also repeated ~15% of the control samples to check for error rates and found a 100% concordance rate for the genotyping results of RNASEL 462 and one mismatch for marker RNASEL 541. Both markers were in Hardy-Weinberg equilibrium in the control samples ($P > 0.05$).

***Statistics.*** For each single nucleotide polymorphism (SNP), allele frequency was determined for the three ethnic groups individually and the frequencies among the ethnic sample groups were compared using the $\chi^2$ test. The Hardy-Weinberg equilibrium test was done on the control population for both SNPs. To estimate the association between prostate cancer risk and each RNASEL SNP, age-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) were determined using logistic regression models. For the purpose of these calculations, study age among controls was the age at last follow-up, whereas age among cases was the age at cancer diagnosis. All analyses were done using SAS statistical software version 9.1 (SAS Institute) and stratified by ethnicity. All statistical tests were two sided and significance was set at $P < 0.05$. Haplotypes and measures of linkage disequilibrium between the two markers were determined using Haploview version 3.2[6] (36) for each race/ethnicity.

## Results

***Allele frequencies.*** We determined the allelic frequency for the Arg[462]Gln and Asp[541]Glu SNPs from 1,539 individuals (648 cases and 891 controls) enrolled in the San Antonio Center for Biomarkers of Risk of Prostate Cancer cohort, including 933 non-Hispanic Caucasian men (503 controls and 430 cases), 392 Hispanic Caucasian men (242 controls and 150 cases), and 214 African American men (146 controls and 68 cases; Table 2). Allelic frequencies for the Arg[462]Gln SNP are significantly different among the Hispanic Caucasians and African Americans ($P$ = 0.01 and 0.0005, respectively; Table 2). The G allele was the most common allele found for the Arg[462]Gln SNP across all ethnic/racial groups. Conversely, the G allele of the Asp[541]Glu SNP was more prevalent among the non-Hispanic Caucasian men, whereas the T allele was more common among Hispanic Caucasian men and African American men (Table 2).

[6] http://www.broad.mit.edu/mpg/haploview/

Our control population was, on average, younger than our prostate cancer cases ($P < 0.0001$). Mean age (SD) for the control group was 61.8 (8.9) years, whereas mean age (SD) for our cases was 65.5 (8.3) years (Table 1). Because of this difference across our two groups and the fact that prostate cancer risk increases with age, all the ORs were adjusted for age. The markers were determined to be in Hardy-Weinberg equilibrium in the control population.

***Associations of RNASEL 462 and 541 SNPs with prostate cancer risk.*** Age-adjusted logistic regression analysis stratified by ethnicity showed a statistically significant association between the AA genotype of Arg[462]Gln and prostate cancer risk in Hispanic Caucasian men, with a >4-fold increase in prostate cancer risk (OR, 4.43; 95% CI, 1.68-11.68; $P$ = 0.003) compared with the GG genotype (Table 3). Furthermore, a >10-fold increase in prostate cancer risk was observed for the AA genotype at Arg[462]Gln in the African American samples (OR, 10.41; 95% CI, 2.62-41.40; $P$ = 0.001; Table 3). In the non-Hispanic Caucasian men, however, no significant association for the Arg[462]Gln variant could be found. Assuming a recessive model, age-adjusted ORs for the presence of the AA genotype in the RNASEL 462 SNP, compared with GG and AG genotypes, showed that the observed risk estimate is slightly decreased in both sample groups (OR, 4.03; 95% CI, 1.56-10.42 in Hispanic Caucasian men, $P$ = 0.004; OR, 9.84; 95% CI, 2.51-38.54 in African American men, $P$ = 0.001; Table 3). In the African American men, we also noticed a significant result under the dominant model (AA/AG versus GG genotypes), with a 2-fold increase in risk estimate (OR, 2.07; 95% CI, 1.06-4.05; $P$ = 0.03; Table 3).

Association analysis of the RNASEL 541 SNP with prostate cancer in age-adjusted samples from the three different ethnic groups revealed that under the assumption of a recessive model, Hispanic Caucasian men with a GG genotype showed a slightly higher risk for prostate cancer (OR, 1.91; 95% CI, 1.16-3.14; $P$ = 0.01; Table 4). No significant association was found in the non-Hispanic Caucasian or African American men for this variant.

The effect of the 462 variant on prostate cancer was calculated using the population attributable fraction where population attributable fraction = $F$ (RR - 1) / RR in which $F$ equals the proportion of cases with mutated allele (0.313 for Hispanic Caucasians and 0.246 for African Americans) and RR equals the relative risk (estimated here with the conservative OR of 1.5; ref. 37). This gives a population attributable fraction of 0.10 for Hispanic Caucasians and of 0.08 for African Americans, indicating that the mutated allele of the 462 variant

**Table 3.** Age-adjusted ORs for RNASEL 462 SNP and prostate cancer risk

| | Genotype | Cases, *n* (%) | Controls, *n* (%) | OR (95% CI) | *P* |
|---|---|---|---|---|---|
| Non-Hispanic Caucasians | | *n* = 430 | *n* = 503 | | |
| | GG | 187 (43.5) | 221 (44) | 1.0 (Reference) | |
| | AG | 183 (42.5) | 225 (45) | 0.98 (0.74-1.30) | 0.89 |
| | AA | 60 (14) | 57 (11) | 1.30 (0.86-1.98) | 0.21 |
| | AA vs AG/GG (Rec A) | | | 1.32 (0.89-1.95) | 0.17 |
| | AA/AG vs GG (Dom A) | | | 1.04 (0.80-1.36) | 0.75 |
| Hispanic Caucasians | | *n* = 150 | *n* = 239 | | |
| | GG | 72 (48) | 136 (57) | 1.0 (Reference) | |
| | AG | 62 (41) | 96 (40) | 1.24 (0.79-1.93) | 0.35 |
| | AA | 16 (11) | 7 (3) | **4.43 (1.68-11.68)** | **0.003** |
| | AA vs AG/GG (Rec A) | | | **4.03 (1.56-10.42)** | **0.004** |
| | AA/AG vs GG (Dom A) | | | 1.45 (0.95-2.22) | 0.09 |
| African Americans | | *n* = 68 | *n* = 145 | | |
| | GG | 45 (66) | 111 (77) | 1.0 (Reference) | |
| | AG | 13 (19) | 31 (21) | 1.26 (0.58-2.73) | 0.56 |
| | AA | 10 (15) | 3 (2) | **10.41 (2.62-41.40)** | **0.001** |
| | AA vs AG/GG (Rec A) | | | **9.84 (2.51-38.54)** | **0.001** |
| | AA/AG vs GG (Dom A) | | | **2.07 (1.06-4.05)** | **0.03** |

Abbreviations: Rec, recessive; Dom, dominant.

is implicated in 10% of the Hispanic Caucasian prostate cancer cases and 8% of the African American prostate cancer cases that we studied. The population attributable fraction or effect of the 541 variant on prostate cancer indicates that the mutated allele is implicated in 17% of Hispanic Caucasian prostate cancer cases of our study group.

***Haplotype analysis of RNASEL 462 and 541 SNPs with prostate cancer risk.*** There was high linkage disequilibrium between the two polymorphisms in the three ethnic/racial sample groups with D-prime values >0.90 in both the Hispanic Caucasians and non-Hispanic Caucasians, indicating that both SNPs are in nearly complete linkage disequilibrium in these sample groups. The D-prime value in the African Americans was 0.79. In the non-Hispanic Caucasians, both SNPs are part of a haplotype block as defined by the Haploview program with the option of adopting block definition proposed by Gabriel et al. (38). A common G-T haplotype for the RNASEL 462 and 541 SNP combination was found to occur more frequently in controls compared with cases in African Americans (controls, 0.686; cases, 0.586; $P = 0.04$) but not in non-Hispanic Caucasians (controls, 0.444; cases, 0.448; $P = 0.87$) or in Hispanic Caucasians (control, 0.526; cases, 0.464; $P = 0.08$; Table 5).

## Discussion

Linkage analyses of high-risk prostate cancer families provide convincing evidence that the *HPC1* locus is likely to harbor a prostate cancer susceptibility gene (9). *RNASEL* has been proposed as the putative tumor suppressor gene for this region through a positional cloning and candidate gene approach (28). Association analysis of two variants within RNASEL ($Arg^{462}Gln$ and $Asp^{541}Glu$) indicated that the results are controversial, and several of the studies have failed to reveal

**Table 4.** Age-adjusted ORs for RNASEL 541 SNP and prostate cancer risk

| | Genotype | Cases, *n* (%) | Controls, *n* (%) | OR (95% CI) | *P* |
|---|---|---|---|---|---|
| Non-Hispanic Caucasians | | *n* = 430 | *n* = 484 | | |
| | TT | 100 (23) | 91 (19) | 1.0 (Reference) | |
| | GT | 190 (44) | 254 (52) | 0.71 (0.51-1.01) | 0.06 |
| | GG | 140 (33) | 139 (29) | 0.95 (0.66-1.38) | 0.80 |
| | GG vs GT/TT (Rec G) | | | 1.21 (0.91-1.60) | 0.20 |
| | GG/GT vs TT (Dom G) | | | 0.80 (0.58-1.10) | 0.17 |
| Hispanic Caucasians | | *n* = 150 | *n* = 242 | | |
| | TT | 41 (27) | 69 (28) | 1.0 (Reference) | |
| | GT | 66 (44) | 125 (52) | 0.88 (0.53-1.47) | 0.63 |
| | GG | 43 (29) | 48 (20) | 1.76 (0.98-3.19) | 0.06 |
| | GG vs GT/TT (Rec G) | | | **1.91 (1.16-3.14)** | **0.01** |
| | GG/GT vs TT (Dom G) | | | 1.11 (0.69-1.78) | 0.67 |
| African Americans | | *n* = 68 | *n* = 146 | | |
| | TT | 31 (46) | 71 (49) | 1.0 (Reference) | |
| | GT | 28 (41) | 60 (41) | 1.02 (0.54-1.94) | 0.94 |
| | GG | 9 (13) | 15 (10) | 1.69 (0.65-4.41) | 0.29 |
| | GG vs GT/TT (Rec G) | | | 1.67 (0.67-4.16) | 0.27 |
| | GG/GT vs TT (Dom G) | | | 1.14 (0.63-2.07) | 0.66 |

**Table 5.** Estimated frequencies for common haplotypes

| | Non-Hispanic Caucasians | | | Hispanic Caucasians | | | African Americans | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cases ($n$ = 430) | Controls ($n$ = 503) | $P$ | Cases ($n$ = 150) | Controls ($n$ = 242) | $P$ | Cases ($n$ = 68) | Controls ($n$ = 146) | $P$ |
| RNASEL 462-541 | | | | | | | | | |
| G-T | 0.448 | 0.444 | 0.87 | 0.464 | 0.526 | 0.08 | 0.586 | 0.686 | **0.04** |
| A-G | 0.344 | 0.346 | 0.93 | 0.284 | 0.232 | 0.10 | 0.168 | 0.197 | 0.47 |
| G-G | 0.201 | 0.209 | 0.69 | 0.223 | 0.239 | 0.60 | 0.175 | 0.114 | 0.08 |

an association. The $Arg^{462}Gln$ AA genotype has been associated with both increased prostate cancer in U.S. Caucasian sample groups (31, 32) as well as decreased prostate cancer risk in Caucasian and Japanese sample groups (33, 34). These findings were in contrast to other studies concluding that the 462 variant was not associated with prostate cancer disease risk in different sample groups including Caucasians from the United States, Sweden, or Germany and Japanese samples (17, 29, 35, 39). The reported results might be conflicting given potential genetic differences in prostate cancer across ethnic and racial groups (5, 6). Other possible explanations for the observed differences might be the manner in which controls were selected for these studies and/or the lack of power to detect association due to small sample sizes.

Previous studies using the $Asp^{541}Glu$ variant within RNASEL indicated that the GG genotype was associated with an increased risk for prostate cancer in a Japanese study (34). On the other hand, a study in European-American samples resulted in a significant positive association of the TT genotype with prostate cancer (17) and in a significant negative association of the TT genotype with prostate cancer in Swedish Caucasian samples (35). No association for the $Asp^{541}Glu$ variant was found in several other studies (29, 31, 33, 39). Furthermore, no study reported to date has examined/confirmed the role of RNASEL variants in the Hispanic Caucasian or African American population.

To test the hypothesis that RNASEL sequence variants are associated with prostate cancer risk, we did a case-control genotype analysis on two common variants of RNASEL in more than 1,500 men from the South Texas region including 933 non-Hispanic Caucasians, 214 African Americans, and 392 Hispanic Caucasians. We included African Americans and Hispanic Caucasians in the analysis because these ethnic study groups are of particular interest; African Americans have the highest risk and death rate, whereas Hispanics are the fastest growing minority population in the United States. The two RNASEL variants analyzed in this study have not been extensively evaluated in African American populations and have not been studied at all in Hispanic Caucasian populations.

The allelic frequencies for $Arg^{462}Gln$ are significantly different among the Hispanic Caucasians and African Americans. This suggests an ethnic-specific allele distribution and is a likely explanation why substantial differences in the incidence of prostate cancer are observed among populations.

The most significant finding was the association of the $Arg^{462}Gln$ genotype with increased prostate cancer risk in both the Hispanic Caucasian and African American samples. Age-adjusted ORs for Arg/Gln (AG) and Gln/Gln (GG) genotypes, compared with Arg/Arg (AA), showed that the Arg/Arg (AA) genotype increases prostate cancer risk over 4-fold in Hispanic Caucasians and over 10-fold in African Americans, which suggests a recessive model for the RNASEL 462 AA genotype. This is to our knowledge the first report on the significant association of $Arg^{462}Gln$ genotypes with increased prostate cancer risk in Hispanic Caucasian or African American men. Our results support the findings of Casey et al. (31) and Xiang et al. (32) showing that the AA genotype of the Gln462 variant is significantly associated with prostate cancer, although they differ from the findings of Casey et al. (31) in that we found the association in Hispanic Caucasians and African Americans but not in non-Hispanic Caucasians. Our results suggest that the role of the $Arg^{462}Gln$ variant in the development of prostate cancer is different across populations. From our sample group, we conclude that the genetic influence of the $Arg^{462}Gln$ variant within RNASEL on prostate cancer in the non-Hispanic Caucasian samples is relatively small, if there is any effect at all. Because it has been shown that the Gln462 AA genotype has three times less enzymatic activity than the wild-type protein (31), our data support the hypothesis that the less active RNASEL protein could leave viral infections intact, leading to inflammation, which eventually could lead to prostate cancer. Additional functional evidence for this variant's role in prostate cancer development comes from the observation that the $Arg^{462}Gln$ variant reduced the ability of RNASEL to cause apoptosis in response to activation by 2-5A (32) and suppresses antiviral effects of IFN (25–27). Furthermore, a strong association between infection with the xenotropic MuLV-related (XMRV) virus and homozygous mutant (Gln462 AA genotype) cases has been reported by Urisman et al. (40), which implicates that defects in RNASEL activity may lead to persistent viral infection *in vivo*.

Analysis of the RNASEL $Asp^{541}Glu$ variant in the three racial/ethnic groups revealed a statistically significant increase in the risk for developing prostate cancer for the RNASEL 541 Glu/Glu (GG) genotype versus the combined Asp/Asp and Asp/Glu genotypes in the Hispanic Caucasian samples. An association of the GG genotype at RNASEL 541 with a slightly increased prostate cancer risk was also reported by Noonan-Wheeler et al. (17) among Caucasian individuals. However, we observed the finding in the Hispanic Caucasian group, whereas non-Hispanic Caucasians did not show a significant positive association for the GG genotype. Our data suggest that susceptibility to develop prostate cancer at this variant is likely ethnic specific and that the RNASEL $Asp^{541}Glu$ variant does not seem to have a major effect on the development of prostate cancer in our non-Hispanic Caucasian or African-American population, whereas it seems to play a role in the Hispanic Caucasian cancer cases. Alternatively, because the $Asp^{541}Glu$ variant had similar enzymatic activity as wild-type RNASEL (32), the substitution of the amino acid Glu by Asp might not

be of any functional significance and it is therefore possible that the RNASEL 541 variant may be in linkage disequilibrium with a nearby functional polymorphism(s) within the *RNASEL* gene or within another gene nearby such that the actual causal variant(s) resides on diverse haplotypes in different study populations. Therefore, additional studies are needed to confirm and clarify the functional significance of these findings in the vulnerability/etiology of prostate cancer.

A common G-T haplotype for the combination of the RNASEL 462 and 541 variants was found to occur more frequently in controls compared with cases in African Americans but not in non-Hispanic Caucasians or in Hispanic Caucasians. These findings are consistent with the observation of Wiklund et al. (35) who found that in sporadic cancer cases, the frequency of the haplotype significantly associated with prostate cancer risk (containing the G-T alleles for RNASEL 462 and 541, respectively) also occurred at higher frequencies among controls compared with sporadic prostate cancer cases.

In conclusion, we confirm the likely involvement of RNASEL in the etiology of prostate cancer and we further provide the first evidence for an association of the *RNASEL* gene with prostate cancer in Hispanic Caucasian and African American men. The prostate cancer risk differs widely between racial/ethnic groups, indicating that race/ethnicity plays a role in the development of prostate cancer. This is likely because each individual brings with them genetic material that sets each race and ethnicity apart. Furthermore, there may be different exposures to environmental factors between the populations. Involvement of environmental factors combined with genetic background may result in the differences in incidence of prostate cancer observed in these populations.

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## Appendices – cont.

### C: Chronic Disease Epidemiology Class Proposal

**PRMD 6636: Chronic Disease Epidemiology**
**Spring Semester 2007**

**A Proposal for:**
**A Life-Course Analytical Approach for Understanding Early Exposure to Androgens and Risk for Prostate Cancer: A Case-Control Study in a Multiethnic Cohort from South Texas**

**Kathleen C. Torkko, PhD, MSPH, MS**

AIMS & HYPOTHESES

Prostate cancer is a disease that is influenced by levels of androgen. Although not conclusively proven, the androgens testosterone and the more bioactive form dihydrotestosterone are thought to be important in the development and growth of prostate tumors. A life-course approach may help improve the understanding of the relationship between androgens and prostate cancer risk given that: 1) prostate tumors may develop a decade or two before the cancer becomes clinically evident, and 2) androgen exposure earlier in life may be more relevant to disease development and growth than androgen levels measured later in life. The goal of this project is to measure surrogates of early and continuing androgen exposure in an existing case-control study and to determine if these exposures are related to risk for prostate cancer. This study will use men enrolled in the SABOR (San Antonio Biomarkers of Risk for Prostate Cancer) study run out of the University of Texas Health Sciences Center, San Antonio. The SABOR study enrolled cohorts of men with and without prostate cancer from south Texas. The control group is undergoing annual screenings for prostate cancer and the study includes White Non-Hispanic, White Hispanic, and African American men.

**AIM 1: Develop and test a questionnaire to assess physical events associated with timing of puberty and hair development, loss, and pattern of hair loss**. A Medline search will be used to identify existing survey instruments. Authors will be contacted for access to instruments. A questionnaire will be designed and tested in a sample of the SABOR population. The final instrument will be translated into Spanish.

**AIM 2: Using the questionnaire developed in AIM 1, information on early androgen exposure will be collected from in men in enrolled in the SABOR study.** To ensure higher participation, multiple mailings will be done and will also be administered to non-responding men appearing for their annual study visits.

> Hypothesis 2A: Men with prostate cancer will have earlier puberty, earlier hair loss, and vertex patterns of hair loss compared to men without cancer.
> Hypothesis 2B: This effect will be stronger in African American men and White Hispanic men.
> Hypothesis 2C: In men with prostate cancer, those with later onset of puberty, balding, and a more frontal pattern of balding will have higher grade disease compared to those with earlier onset.

BACKGROUND & SIGNIFICANCE

Prostate cancer is the most commonly diagnosed non-skin cancer and one of the ten leading causes of death in American men.1 The American Cancer Society estimates that over 218,000 new cases will be diagnosed this year with 27,050 men dying from the disease making it the second leading cause of

death from cancer after lung.2 Age and race/ethnicity and remain the two strongest risk factors for prostate cancer. In 1999-2003, the median age of diagnosis was 683 and the more current estimate of prostate cancer incidence is 924.6 (per 100,000) in men 65 and over and 58.5 in men under 65; the rate is 0.7 in men 30-39.4 The incidence for prostate cancer is much higher in African Americans (255.5) compared to White Non-Hispanic men (166.6) and all other racial/ethnic groups. African Americans are also at increased risk for dying compared to Whites (mortality rate 62.3 and 21.7, respectively).

The etiology of prostate cancer is not well known, although both genetic and environmental factors are believed to play a role. A twin study from Scandinavia estimated that 42% of the risk for prostate cancer might be explained by heritable factors.5 A diverse range of foods and nutrients have been found to moderately affect risk for prostate cancer, including soy, isoflavones, milk, saturated fats, and tomato products.6 Higher birth weight and length may be associated with more aggressive disease.7 Higher BMI and adult weight gain increase risk of dying from prostate cancer8 and aggressive disease is associated with being overweight.9,10. Interestingly, obesity itself is inversely related to risk for prostate cancer in middle-aged men.11 A history of diabetes may be associated with a decreased risk of prostate cancer, especially late stage tumors.12 Many other environmental factors have been studied such as pesticide exposure and red meat consumption, but results have been mixed.

Although prostate cancer diagnosis is strongly associated with increasing age, the initiation of prostate cancer appears to start earlier in adulthood. An autopsy study in 249 men aged 20-69 who died of other causes found evidence of prostate cancer in 2% and 29% percent of prostates from men aged 20-29 and 30-39, respectively.13 Given, as discussed earlier, that diagnosis of prostate cancer occurs a decade or more later, this argues for a life-course approach to studying exposures associated with risk for the disease.

Prostate cancer is considered a hormone-dependent malignancy that grows from androgen-dependent tissue. The androgen testosterone and its bioactive form, dihydrotestosterone (DHT), have been shown to be necessary for the normal growth and development of the prostate, and epidemiologic evidence implicates their role in the etiology of prostate cancer.14,15,16,15,17 Androgens are involved in controlling the growth of androgen-sensitive malignant prostate cells and levels of estrogen may influence their transformation to a malignant phenotype.18 The Prostate Cancer Prevention Trial demonstrated that men given the a drug that blocks the metabolism of testosterone had a 24.8% reduction in cancer prevalence over seven years compared to men given placebo.19

Epidemiologic studies have failed to consistently show that circulating androgens are associated with prostate cancer risk. At least one prospective study has found an association with higher levels of serum testosterone and increased prostate cancer risk.20 In Caucasians, prostate cancer was associated with the ratio of total testosterone to total estradiol, but not to absolute levels of the sex hormones.21 Animal studies have shown that androgens are strong tumor promotors for carcinogenesis even at very low concentrations.22 This might explain why it has been difficult to prove associations of elevated serum testosterone levels with risk for prostate cancer. It might also be that early and long-term androgen exposure may have more impact on prostate cancer growth and that blood measurements later in life may be affected by age confounding.23 This observation again supports a life-course approach to the study of androgen exposure and risk for prostate cancer.

It is the aim of this study to assess surrogate measures of early and continuing androgen exposure and determine how these are related to risk of prostate cancer in a group of White Non-Hispanic, White Hispanic, and African American men from south Texas participating in a mixed cohort/case-control study of prostate cancer. Because androgens play an important role in both puberty24 and hair growth and loss25,26, a questionnaire will be used to assess timing of different exposures including age at shaving initiation, age of hair loss initiation, and hair loss pattern (i.e., frontal vs. vertex). Serum free testosterone levels are strongly associated with baldness.27 Male pattern baldness that often occurs decades before a prostate cancer diagnosis was found to be a risk factor for clinical prostate cancer.28 Vertex balding is associated both with prostate cancer (OR=1.54; 1.19, 2.00; no association found with frontal balding) and with high grade cancer (in men 60-69: OR=2.91; 1.59, 5.32).29 The odds ratio for frontal balding and high grade cancer was 1.80 (1.02, 3.16). A large Kaiser Permanente study found that younger age at

shaving initiation (<14 years old) was associated with a modest risk for prostate cancer (OR=1.49; 95%CI: 1.01,2.22), but only in non-white men.30 The main hypothesis of this study is that men with prostate cancer will have earlier puberty, earlier hair loss, and vertex patterns of hair loss compared to men without cancer and that this relationship will differ by racial/ethnic group.

A secondary hypothesis involves an interesting conundrum in the story of androgens and prostate cancer. Although higher levels of testosterone may be associated with increased risk for prostate cancer, low levels appear to be associated with more aggressive prostate cancer. Men with low total testosterone were more likely to have positive surgical margins on their radical prostatectomy specimens31 and more advanced pathological stage or aggressive disease.32,33 Two recent Japanese studies demonstrated that low testosterone is associated with higher Gleason grade.34,35 Therefore, we hypothesize that in men who have prostate cancer, those with later onset of puberty, balding, and a more frontal pattern of balding will have higher grade disease compared to those with earlier onset.

MATERIALS AND METHODS

Study Design. The proposed study will use a cross-sectional study design in an existing cohort/case-control study on prostate cancer. A questionnaire will be developed to assess early androgen exposures in a selection of prostate cancer cases and controls.

Study Population. Study participants will come from the population-based prospective SABOR cohort study (San Antonio Biomarkers Of Risk for prostate cancer) and its sister study that enrolls existing prostate cancer cases from the same clinics that enroll SABOR patients. Both studies are run through the University of Texas Health Sciences Center, San Antonio (UTHSCSA).36 SABOR began enrolling men in May 2001 to examine differences in risk for prostate cancer by race/ethnicity. To date, approximately 3,000 men have enrolled in the studies. Three racial/ethnic groups reflecting the diversity of the southern Texas population are enrolled: non-Hispanic Whites (NHW), Hispanic Whites (HW), and African Americans (AA). Race is self-identified and Hispanic ethnicity is assigned using the Hazuda model for the identification of Mexican Americans and other Hispanic ethnicities.37 The Hispanic population of south Texas is approximately 95% Mexican American. Table 1 gives numbers of cases and controls by race/ethnicity. All appropriate approvals for the consent form and survey instrument will be sought according to UTHSCSA IRB rules and regulations.

Table 1. Current number so participants in the SABOR studies (as of 3/31/07)

| Race/Ethnicity | Total* | Cancer |
|---|---|---|
| Non-Hispanic White | 1,578 | 495 |
| Hispanic White | 1,052 | 180 |
| African American | 471 | 69 |

* Not all men without cancer will be eligible to be controls due to high PSA or abnormal DRE exam

All men with prostate cancer will be eligible to participate. Cases in this analysis will have histologically-confirmed prostate cancer. Gleason scores will be determined from chart reviews and physician reports. High-grade cancers will defined as men with Gleason scores of seven or greater. Prostatectomy scoring will be used preferentially over biopsy scores when available.

Controls, selected from the SABOR cohort, will be eligible for this analysis if they have prostate-specific antigen (PSA) values less than 2.5 ng/ml at all visits (up to six annual visits) and a normal digital rectal exam (DRE) at all visits. Age in the study will be defined as age at diagnosis for the cases and age at last study visit for the controls. Controls will be selected to match cases on age in 5-year age groups.

Questionnaire. Authors with published papers on questionnaires that survey baldness[38], and measures of puberty (*e.g.*, age a shaving initiation, age of pubic and/or chest hair appearance)[30] will be contacted for access to established and validated instruments. Questions will be edited to meet the needs

of the proposed study and to address study hypotheses. The questionnaire will be tested in a small group of SABOR participants who are not eligible to participate in the proposed study (*i.e.*, abnormal DRE exam with no subsequent cancer diagnosis). Questions will be modified as necessary. The final questionnaire will be translated in to Spanish and both the English and Spanish versions will be sent to the UTHSCSA IRB office for approval. The survey will be mailed at least twice to eligible SABOR participants. Men who attend annual study visits and who have not yet returned a questionnaire will be given the opportunity to complete the questionnaire during their visit. Data will be cleaned and verified by a duplicate entry process.

Amount and type of baldness will be assessed using the Norwood-Hamilton Baldness Scale (Figure 1).[39] Men will indicate the pattern that most closely matches their current level and type of baldness and also at what age they first noticed hair loss. Although it is recommended that male balding patterns be assessed by trained personnel, men's self-assessment both currently and retrospectively can be adequate.[40] For analytic purposes, subjects will be grouped according to the degree and pattern of baldness as: no baldness (Type I), frontal baldness (Types II & III), and vertex baldness (Types III Vertex, IV-VII). To validate this approach, a research nurse will independently assess participants during their next annual visit to determine agreement between self-assessment by the participant and assessment by trained study personnel. The baldness groups may be dichotomized by pattern only as normal/ frontal (Types I-III) versus vertex pattern (Types III Vertex, IV-VII).

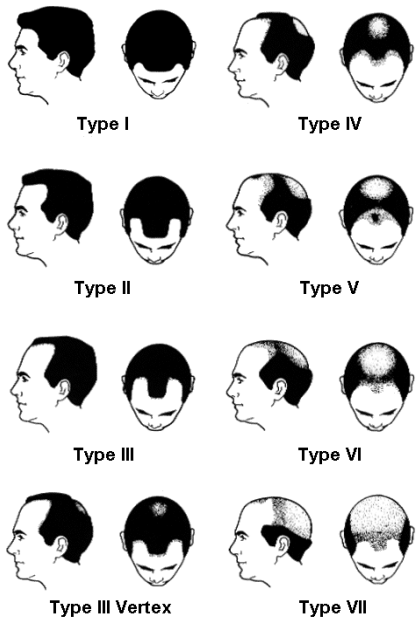

Figure 1. Norwood Hamilton Baldness Scale.

Potential Biases. A potential pitfall of the proposed study design is recall bias and exposure misclassification. Men are being asked to remember events that occurred several decades ago. Recall bias may be less of a problem because there is little reason to assume that men with cancer will recall events differently from those without cancer, especially if cases and controls are age-matched. To help men remember ages that balding or shaving started, prompts will be used to help them recall. For example, a man can be asked if he was shaving by the time he entered high school or if he was balding at the time his children were born. Men may be asked to bring pictures to clinic visits showing hair maintenance or loss at various ages.

Statistical Analyses. Other covariates that are collected as part of usual SABOR study activities will be included in the analyses. These include age, height, weight, BMI, and current serum testosterone levels.

An additive scale of early androgen exposures will be tested. For example, balding pattern can be scored as 1 point for vertex pattern and 0 points for none/frontal pattern. For age of hair loss, before age 30 could equal 1 point and >30 = 0 points. For shaving initiation, before age 14 equals 1 point and after age 14 equals 0 points. This score will be tested in the logistic model. If a score is validated, this will become the main variable of interest in the logistic model.

All analyses will be stratified by ethnicity. Univariate associations with prostate cancer will be tested using ANOVA or t-tests (or equivalent non-parametric tests), chi-square tests, and logistic regression analyses. Variables that have a p value <0.1 will be tested in the full logistic model. P-values <0.05 will be considered statistically significant. Analyses will be completed using SAS 9.1 statistical software (SAS institute, Inc., Cary, NC).

Timeline: It is proposed that the study will take two years to complete. Design and development of the survey instrument should occur in the first 6 months, including validation, Spanish translation and IRB approval. Survey mailings and administration will occur during the next 12-15 months (to allow for follow-up and measurements during annual study visits). Data entry, cleaning, and preliminary analysis can begin during this period. Final data cleaning, analysis and paper preparation can be done in the last 3-6 months of the 2-year grant.

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**Appendices – cont.**

**D: Transcript**

University of Colorado Health Sciences Center
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EPORT DATE: 03/27/2008

KATHLEEN CARROLL TORKKO
YOUR STUDENT NUMBER: XXX-XX-5050

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============================================================
     COURSE TITLE          CRSE NR          HRS GRADE      PNTS
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------ SPRING SEM 2007   UCDHSC - HEALTH SCIENCES       ------
     NON-DEGREE                        NONDEGREE PUBLIC HEALTH
 CHRNIC DISEASE EPIDEMLGY PRMD 6636       2.0  A          8.0
ATT   2.0  EARNED   2.0  GPAHRS   2.0  GPAPTS   8.00 GPA 4.000

------ FALL SEM 2007     UCDHSC - HEALTH SCIENCES       ------
     NON-DEGREE                        NONDEGREE PUBLIC HEALTH
 ANALYTIC METHODS IN EPI  PRMD 7915       1.0  A          4.0
ATT   1.0  EARNED   1.0  GPAHRS   1.0  GPAPTS   4.00 GPA 4.000
------------------------------------------------------------
                      CUMULATIVE CREDITS:
            TR HRS   CU HRS  TOT HRS  QUAL HRS  QUAL PTS   GPA
GNON  SEM     0.0      3.0      3.0       3.0     12.00   4.000
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*** END OF ACADEMIC RECORD ***

**Appendices – cont.**

**E: HSBC 4001/5001 Introduction to Epidemiology Syllabus, Spring 2008**

UNIVERSITY OF COLORADO AT DENVER AND HEALTH SCIENCES CENTER  COLLEGE OF LIBERAL ARTS & SCIENCES

**HBSC 4001 / 5001: INTRODUCTION TO EPIDEMIOLOGY**

Term: Spring 2008
Course dates/times: Tuesdays, 4-6:50 p.m.

Course location: (WC)159
Office Hours: By appointment 1 hour before class
Administration Building 255B

Professor: Kathleen C. Torkko, PhD, MSPH
Office location: Anschutz Medical Campus;
RC1-North, Room P18-5120
Phone: 303-724-3063
Email address: kathleen.torkko@uchsc.edu
Web site and/or BlackBoard site

**Catalogue Description (HBSC 5001):**

Introduces the basic concepts of public health and epidemiology, including assessment of disease in the community, the study of causation and association of disease with lifestyle and environmental risk factors, as well as related special topics. Prereq: upper division standing and course in basic statistical methods. Cross-listed with HBSC 4001.

**Instructor Description:**

This is an introductory epidemiology course designed for graduate students in the Health and Behavioral Sciences (HBS) program at the University of Colorado Denver. The model for this course is the Introduction to Epidemiology (PRMD 6630) taught in the Department of Preventive Medicine and Biometrics (PMD) at the Health Sciences Center campus. This course will cover the same basic epidemiologic concepts taught in that class allowing students to take advanced epidemiology courses taught through PMD. Because epidemiology is considered part of the medical sciences and its roots come from the study of infectious disease, it is necessary to discuss the medical aspects of disease to illustrate many epidemiologic principles. It will also be necessary for students to brush up on their basic math skills. Content for this course will include some emphasis on topics that may be of more interests to HBS students as compared to the more purely medical focus of the 6630 course.

This course will provide students with an understanding of the basic methods and tools used by epidemiologists to study rates and risks for disease and other factors that affect the health of people. Epidemiologic techniques are used to study a wide variety of health concerns including infectious disease outbreaks, risk factors for chronic diseases, and societal and behavioral factors affecting access to and use of health services. This variety makes epidemiology an exciting and useful area of study. Although this course will not turn you into epidemiologists, I hope you will develop some excitement for the subject and an appreciation for the relevance of epidemiology to your areas of interest.

Epidemiology is not black-and-white. Often there is not necessarily a "right" answer. There may be many ways to study a problem and the choice of an approach will depend on the nature of the questions being asked and on such practicalities as the availability of data and costs. Sometimes we choose the best answer or one way to study a problem, although it is not

necessarily the only answer nor the only way to study it. Epidemiology is often a science of compromises. This can be particularly aggravating for students who might prefer that all questions have either right or wrong answers. All this can make epidemiology a difficult subject to teach and to learn. It is possible that you may pose questions that I am not able to answer immediately, or I may change my mind after further reflection. I also expect that some of you will come up with answers that had not occurred to me. I anticipate a dialog between you and myself. Please feel free to ask questions. I look forward to teaching AND to learning from you.

One theme for this class is the use of epidemiologic techniques to study health disparities in populations. You will be expected to complete a final project consisting of a short paper using what was learned over the semester to describe a health disparity whether by race, gender, age, geography, socioeconomic status or other factors of interest. I have a grant to study health disparities in cancer, particularly in prostate cancer. This is a wonderful opportunity for students to teach the teacher about health disparities, particularly in prostate cancer.

To learn epidemiology, a student may need several passes through the material. It is expected that you will have read all materials and performed all tasks assigned for a particular session prior to the start of class. Reading the material in advance will help you formulate questions. My teaching style will be interactive with in-class exercises and self-assessments to facilitate in-class discussion to help me gauge now well students are learning (and how well I am explaining things!).

Because we are meeting for 3 hours, the class session will be divided into two sub-sessions, A and B, with a short break in between depending on time constraints for a particular lecture. Each session will include two separate lectures on related topics or a lecture with an in-class exercise. Much of the in-class work will require some preparation that will serve as the homework for the (sub-)session.

Handouts of the lecture slides will be posted at least 24 hours prior to each lecture so you may print them for lecture notes. Materials will be accessible on Blackboard. I will be available before each session for questions and additional help. I will try to arrive at least 30-60 minutes prior to each class session (I am a person who is usually running late!). I can make it earlier to class by appointment. Please feel free to e-mail me or call.

**Course Objectives:**

At the end of this course, the student will:

1. Be able to use epidemiologic terminology
2. Understand and calculate different rates and measures of association (*i.e.,* OR, RR)
3. Articulate clearly the strengths and limitations of different epidemiologic study designs
4. Understand important epidemiologic concepts including confounding, bias, and causation
5. Be able to critically read epidemiologic literature to recognize study design and analytical strengths and limitations.

**Required Text**:

Gordis L. Epidemiology, 3rd Ed., Elsevier Saunders, 2004

Assignments:

Homework: Generally homework will be assigned for each sub-session. This includes working out problems, providing short answers and definitions, and reading assignments. The homework assigned will usually cover material that will be discussed at the session. Although

this may seem counter-intuitive, grappling with problems and deriving your own solutions before learning how other people have done it will give you experience in solving new problems and allow you to develop a clearer view of the strengths and weaknesses of accepted solutions. Try working out problems first by yourself. If you run into difficulty, feel free to collaborate with your fellow students. But don't just copy answers. If you really don't understand something, discuss it or contact me. Homework must be submitted prior to class electronically or on hard copy at the beginning of class. Failure to do so will deduct 20% from your score (unless you have an EXCELLENT reason for being late). Graded assignments will be returned the next class session. Answers to homework will be posted on Blackboard a week after they are due.

Exams: There will be two formal exams, a midterm (on March 11) and a final (on May 12). The exams will be in-class and open book and will include multiple choice questions and short answers. Some calculations will be required so calculators will be permitted, but not computers. The midterm will cover material presented up to that point; the final will cover the entire term with an emphasis on the latter half. There will be opportunities for formal review before each exam. The first hour of the session will be given to any review questions with the latter 2 hours for the exam.

A final project will entail writing a 3-5 page paper (double-spaced) plus tables or figures. The topic will be of your choice but must cover a health disparity in Colorado, the US, or elsewhere around the world. The topic must be OK'd by me (topic must be chosen by April 1). Preference should be given to cancer, particularly prostate cancer, or another topic that is of great interest to you or your work. There will be no preferential grading given to those who pick prostate cancer, so you are free to choose as you wish. If you can't decide on a topic, I will assign one to you. For this project you will use epidemiology to describe the disparity (rates, risks, etc.) and discuss the types of studies, source of data to describe the disparity. You will briefly discuss any potential problems with the data or gaps in our knowledge. We will discuss the requirements in more detail during a class session. The paper must be submitted electronically or on hard copy by May 6.

Graded midterms will be returned the following class session. Graded final exams and projects will be available at the HBS office after May 19th.

Grades:

Final grades will be determined on a curve and based on homework assignments, in-class exercises, two exams (midterm and final), and a final project according to the following distribution:

| | |
|---|---|
| Homework | 15 % |
| In-class exercises | 15 % |
| Midterm Exam | 25 % |
| Final Exam | 25 % |
| Final Project | 20 % |
| Total | 100% |

In-class participation will also be assessed by awarding additional points based on a scale from 0-10 with "0" meaning you never opened your mouth in class to 10 meaning you participated in most if not all discussions. This means a total score of 110 points is possible, but remember, the class is graded on a curve.

Course Policies:

Class attendance and participation is essential for success. No deductions in the final grade will be applied for non-attendance (as long as assignments are turned in on time), but you will miss out on critical questions and discussions. There is no requirement to notify me if you miss class, but I would appreciate a courtesy e-mail to explain unanticipated absences.

The schedule of coursework listed below is not written in stone and may be subject to unplanned changes such as instructor or guest lecturer illness. Additionally, I reserve the right to change the syllabus depending on the needs and interests of the students. Students will be given appropriate, timely, and written notification of any changes.

Homework can either be (clearly) handwritten or typed with room in the margins for me to make comments. Homework can be submitted electronically (MS-Word) or on hard copy. When students' work conveys that they require additional help in composition or math, students will be referred to the Writing Lab and/or the Math Lab. It is your responsibility to clarify missed assignments with me. Homework not submitted in time (by the beginning of the class session) will have a 20% reduction applied to the score. Late homework not submitted by or at the beginning of the following class session will not be graded (although you will get feedback).

If you will miss a scheduled exam, you must notify me prior to the start of the exam. In cases of an emergency, you can call me on my cell phone or contact the HBS office to leave a message. A make-up exam will be re-scheduled. This should be done within a week after the date of the original exam. This may mean you will have to travel to the Anschutz Medical Campus to take the exam unless I can find someone to proctor it on the Auraria Campus. If a make-up is necessary, I ask the other students to refrain from sharing any specific information about the content of the exam with the student(s) who will be taking the make-up.

Course Schedule:

| Date | Topic | Required Reading* | Assignments |
|---|---|---|---|
| 01/22/08 A | Introduction, Course Requirements | | |
| 01/22/08 B | Introduction to Epidemiology | Gordis Chapt 1 | None due |
| 01/29/08 A | Measures of Health Status | Gordis Chapt 3 (pp 32-33; 42-46) Chapt 4 (pp 48-58) | Homework 1 |
| 01/29/08 B | Incidence & Prevalence | Gordis Chapt 4 (p 48-58) | Homework 2 |
| 02/05/08 A | Rate Adjustment & Attributable Risk | Gordis Chapt 4 (pp 58-70) Chapt 12 | Homework 3 |
| 02/05/08 B | In-class Exercise | Exercise 1 | Exercise 1 |
| 02/12/08 A | Cohort Studies & Relative Risk | Gordis Chapt 9, Chapt 11 (pp 177-81); *Scand J Pub Health* 2007;35:306-12. | Homework 4 |
| 02/12/08 B | Case-Control Studies & Odds Ratios | Gordis Chapt 10, Chapt 11 (pp 181-88); | Homework 5 |
| 02/19/08 A | Other Observational Study Designs / Causation | Gordis Chapt 14 | Homework 6 |
| 02/19/08 B | In-class Exercise | Exercise 2 | Exercise 2 |

| | | | |
|---|---|---|---|
| 02/26/08 A | Clinical Trials & Prognosis | Gordis Chapt 6, 7, 8 | Homework 7 |
| 02/26/08 B | In-class Exercise | Exercise 3 | Exercise 3 |
| 03/04/08 A | Natural History of Disease / Levels of Prevention | Gordis Chapt 2 | Homework 8 |
| 03/04/08 B | In-class Exercise | Exercise 4 | Exercise 4 |
| 03/11/08 A | Review questions | | |
| 03/11/08 B | MIDTERM EXAM | | |
| 03/18/08 A | Bias, Confounding & Effect Modification | Gordis Chapt 15. *Scand J Pub Health* 2007;35:306 | Homework 9 |
| 03/18/08 B | In-class Exercise | Exercise 5 | Exercise 5 |
| 03/25/08 | SPRING BREAK – no class | | |
| 04/01/08 A | Screening Tests: Sensitivity, Specificity, etc. | Gordis Chapt 5, 18 | Homework 10 |
| 04/01/08 B | In-class Exercise | Exercise 6 | Exercise 6 |
| 04/08/08 A | Guest Lecture: Using GIS in Epidemiology - Thomas | *Environmental Health Perspectives* 2004;112:998-1006 | TBA |
| 04/08/08 B | Prostate Cancer Epidemiology; Discussion of Final Health Disparities Project | *Cancer* 2007;110:1889-99. | Homwork 11 |
| 04/15/08 A | Guest Lecture: Health Disparities in Tobacco Burden – Levinson | TBA | TBA |
| 04/15/08 B | Epidemiology of Health Disparities | *J Transcult Nurs* 2008;19:83-91. | Homework 12 |
| 04/22/08 A | Guest Lecture: Lifecourse Epidemiology - Dablea | *Ann Rev Pub Health* 2005;26:1-25 | TBA |
| 04/22/08 B | Guest Lecture: Community Epidemiology – Baxter | TBA | TBA |
| 04/29/08 A | Guest Lecture: Sun Protection in Children – Crane | TBA | TBA |
| 04/29/08 B | Criticism of Epidemiology: Hormone Replacement Therapy and Heart Disease in Women | Do We Really Know What Makes Us Healthy? By Gary Taubes, The Times Magazine, 9/16/07 | Homework 13 |
| 05/06/08 A | Ethics & Human Subject Research | Gordis Chapt 20 | Homework 14 |
| 05/06/08 B | In-class Exercise | Exercise 7 | Exercise 7 |
| 05/13/08 A | Review Session | | |
| 05/13/08 B | FINAL EXAM | | |

*Lists for other reading assignments (pertinent papers, etc.) will be available the first day of class or throughout the course before specific classes.

## Appendices – cont.

### F: List of Panel of Single Nucleotide Polymorphisms

| SNP_Name | Chromosome | Gene |
|---|---|---|
| rs9332975 | 2 | SRD5A2 |
| rs2268794 | 2 | SRD5A2 |
| rs2268796 | 2 | SRD5A2 |
| rs2208532 | 2 | SRD5A2 |
| rs4952222 | 2 | SRD5A2 |
| rs632148 | 2 | SRD5A2 |
| rs3754838 | 2 | SRD5A2 |
| rs9332960 | 2 | SRD5A2 |
| rs12721364 | 12 | VDR |
| rs9729 | 12 | VDR |
| rs739837 | 12 | VDR |
| rs11168267 | 12 | VDR |
| rs11574077 | 12 | VDR |
| rs2239182 | 12 | VDR |
| rs2107301 | 12 | VDR |
| rs2239179 | 12 | VDR |
| rs12717991 | 12 | VDR |
| rs12721370 | 12 | VDR |
| rs2189480 | 12 | VDR |
| rs3819545 | 12 | VDR |
| rs3782905 | 12 | VDR |
| rs2239186 | 12 | VDR |
| rs2254210 | 12 | VDR |
| rs2238136 | 12 | VDR |
| rs4760648 | 12 | VDR |
| rs11168287 | 12 | VDR |
| rs4328262 | 12 | VDR |
| rs4237855 | 12 | VDR |
| rs11574026 | 12 | VDR |
| rs7302235 | 12 | VDR |
| rs12581281 | 12 | VDR |
| rs4516035 | 12 | VDR |
| rs7139166 | 12 | VDR |
| rs1048691 | 12 | CYP27B1 |
| rs4646537 | 12 | CYP27B1 |
| rs8176345 | 12 | CYP27B1 |
| rs703842 | 12 | CYP27B1 |
| rs4646536 | 12 | CYP27B1 |
| rs2762929 | 20 | CYP24A1 |
| rs8118441 | 20 | CYP24A1 |
| rs6068810 | 20 | CYP24A1 |
| rs6097807 | 20 | CYP24A1 |
| rs2762934 | 20 | CYP24A1 |
| rs1570669 | 20 | CYP24A1 |
| rs2296239 | 20 | CYP24A1 |

| SNP_Name | Chromosome | Gene |
|---|---|---|
| rs6068816 | 20 | CYP24A1 |
| rs4809958 | 20 | CYP24A1 |
| rs3787554 | 20 | CYP24A1 |
| rs2244719 | 20 | CYP24A1 |
| rs2762941 | 20 | CYP24A1 |
| rs2181874 | 20 | CYP24A1 |
| rs4809960 | 20 | CYP24A1 |
| rs2296241 | 20 | CYP24A1 |
| rs2245153 | 20 | CYP24A1 |
| rs2585428 | 20 | CYP24A1 |
| rs13038432 | 20 | CYP24A1 |
| rs6022999 | 20 | CYP24A1 |
| rs2248359 | 20 | CYP24A1 |
| rs4809957 | 20 | CYP24A1 |
| rs1059519 | 19 | PDF/GDF15 |
| rs1059369 | 19 | PDF/GDF15 |
| rs1804826 | 19 | PDF/GDF15 |
| rs16982345 | 19 | PDF/GDF15 |
| rs1227733 | 19 | PDF/GDF15 |
| rs1491711 | 4 | GC/VDBP |
| rs17383291 | 4 | GC/VDBP |
| rs705117 | 4 | GC/VDBP |
| rs2282679 | 4 | GC/VDBP |
| rs7041 | 4 | GC/VDBP |
| rs4752 | 4 | GC/VDBP |
| rs222020 | 4 | GC/VDBP |
| rs1352843 | 4 | GC/VDBP |
| rs3733359 | 4 | GC/VDBP |
| rs16847028 | 4 | GC/VDBP |